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(74) Agents: GOLDMAN, Michael, L. et al.; Nixon Peabody
LLP, Clinton Square, P.O. Box 31051, Rochester, NY
14603-1051 (US).

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(71) Applicant: EDEN BIOSCIENCE CORPORATION
[US/US]; 11816 North Creek Parkway N., Bothell, WA
98011-8205 (US).

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(72) Inventors: SONG, Xiaoling; 14110 NE 179th Street, Apt.
75, Woodinville, WA 98072 (US). FAN, Hao; 19712 6th
Drive S.E., Bothell, WA 98012 (US). WEI, Zhong-Min;
8230 125th Court, Kirkland, WA 98034 (US).

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(54) Title: RECEPTORS FOR HYPERSENSITIVE RESPONSE ELICITORS AND USES THEREOF

(57) Abstract: The present invention is directed to an isolated protein which serves as a receptor in plants for a plant pathogen hypersensitive response elicitor. Also disclosed are nucleic acid molecules encoding such receptors as well as expression vectors, host cells, transgenic plants, and transgenic plant seeds containing such nucleic acid molecules. Both the protein and nucleic acid can be used to identify agents targeting plant cells to enhance a plant's receptivity to treatment with a hypersensitive response elicitor and to directly impart plant growth enhancement as well as resistance against disease, insects, and stress.

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RECEPTORS FOR HYPERSENSITIVE RESPONSE ELICITORS AND USES THEREOF

This application claims benefit of U.S. Provisional Patent Application
5 Serial Nos. 60/191,649, filed March 23, 2000 and 60/250,710, filed December 1,
2000.

FIELD OF THE INVENTION

10 The present invention relates to receptors for hypersensitive response
elicitors and uses thereof.

BACKGROUND OF THE INVENTION

15 Plants have evolved a complex array of biochemical pathways that
enable them to recognize and respond to environmental signals, including pathogen
infection. There are two major types of interactions between a pathogen and plant --
compatible and incompatible. When a pathogen and a plant are compatible, disease
generally occurs. If a pathogen and a plant are incompatible, the plant is usually
20 resistant to that particular pathogen. In an incompatible interaction, a plant will
restrict pathogen proliferation by causing localized necrosis, or death of tissues, to a
small zone surrounding the site of infection. This reaction by the plant is defined as
the hypersensitive response ("HR") (Kiraly, Z. "Defenses Triggered by the Invader:
Hypersensitivity," Plant Disease: An Advanced Treatise 5:201-224 J. G. Horsfall and
25 E. B. Cowling, eds. Academic Press, New York (1980); (Klement "Hypersensitivity,"
Phytopathogenic Prokaryotes 2:149-177, M.S. Mount and G. H. Lacy, eds. Academic
Press, New York (1982)). The localized cell death not only contains the infecting
pathogen from spreading further but also leads to a systemic resistance preventing
subsequent infections by other pathogens. Therefore, HR is a common form of plant
30 resistance to diseases caused by bacteria, fungi, nematodes, and viruses.

A set of genes designated as *hrp* (Hypersensitive Response and
Pathogenicity) is responsible for the elicitation of the HR by pathogenic bacteria,
including *Erwinia* spp, *Pseudomonas* spp, *Xanthomonas* spp, and *Ralstonia
solanacearum* (Willis et al. "*hrp* Genes of Phytopathogenic Bacteria," Mol. Plant-

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- Microbe Interact. 4:132-138 (1991), Bonas, U. "*hrp* Genes of Phytopathogenic Bacteria," pages 79-98 in: Current Topics in Microbiology and Immunology, Vol. 192, Bacterial Pathogenesis of Plants and Animals: Molecular and Cellular Mechanisms. J. L. Dangl, ed. Springer-Verlag, Berlin (1994); Alfano et al., "Bacterial Pathogens in Plants: Life Up Against the Wall," Plant Cell 8:1683-98 (1996).
- Typically, there are multiple *hrp* genes clustered in a 30-40 kb DNA. Mutation in any one of the *hrp* genes will result in the loss of bacterial pathogenicity in host plants and the HR in non-host plants. On the basis of genetic and biochemical characterization, the function of the *hrp* genes can be classified into three groups: 1) structural genes encoding extracellularly located HR elicitors, for example harpin of *Erwinia amylovora* (Wei et al. "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85 (1992)); 2) secretion genes encoding a secretory apparatus for exporting HR elicitors and other proteins from the bacterial cytoplasm to the cell surface or extracellular space (Van Gijsegem et al., "Evolutionary Conservation of Pathogenicity Determinants Among Plant and Animal Pathogenic Bacteria," Trends Microbiol. 1:175-180 (1993); He et al. "Pseudomonas syringae pv. Syringae harpin_{pss}: A Protein that is Secreted Via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255 (1993); Wei et al., "HrpI of *Erwinia amylovora* Functions in Secretion of Harpin and is a Member of a New Protein Family," J. Bacteriol. 175:7985-67 (1993), Arlat et al. "PopA1, a Protein which Induces a Hypersensitive-Like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-53 (1994), Galan et al., "Cross-talk between Bacterial Pathogens and their Host Cells," Ann. Rev. Cell Dev. Biol. 12:221-55 (1996); Bogdanove et al., "*Erwinia amylovora* Secretes Harpin via a Type-III Pathway and Contains a Homolog of yopN of Yersinia," J. Bacteriol. 178:1720-30 (1996); Bogdanove et al., "Homology and Functional Similarity of a *hrp*-linked Pathogenicity Operon, *dspEF*, of *Erwinia amylovora* and the *avrE* locus of *Pseudomonas syringae* pathovar tomato," Proc Natl Acad Sci USA 95:1325-30 (1998)); and 3) regulatory genes that control the expression of *hrp* genes (Wei, Z. M., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85 (1992); Wei et al., "*hrpL* Activates *Erwinia amylovora hrp* Genes in Response to

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Environmental Stimuli," J. Bacteriol. 174:1875-82 (1995); Xiao et al., "A Single Promoter Sequence Recognized by a Newly Identified Alternate Sigma Factor Directs Expression of Pathogenicity and Host Range Determinants in *Pseudomonas syringae*," J. Bacteriol. 176:3089-91 (1994); Kim et al., "The *hrpA* and *hrpC* Operons of *Erwinia amylovora* Encode Components of a Type III Pathway that Secretes Harpin," J. Bacteriol. 179:1690-97 (1997); Kim et al., "HrpW of *Erwinia amylovora*, a New Harpin that Contains a Domain Homologous to Pectate Lyases of a Distinct Class," J. Bacteriol. 180:5203-10 (1998); Wengelnik et al., "HrpG, A Key *hrp* Regulatory Protein of *Xanthomonas campestris* pv. *Vesicatoria* is Homologous to Two Component Response Regulators," Mol. Plant-Microbe Interact. 9:704-12 (1996)). Because of their role in interactions between plants and microbes, *hrp* genes have been a focus for bacterial pathogenicity and plant defense studies.

In addition to the local defense response, HR also activates the defense system in uninfected parts of the same plant. This results in a general systemic resistance to a secondary infection termed Systemic Acquired Resistance ("SAR") (Ross, R. F. "Systemic Acquired Resistance Induced by Localized Virus Infections in Plants," Virology 14:340-58 (1961); Malamy et al., "Salicylic Acid and Plant Disease Resistance," Plant J. 2:643-654 (1990)). SAR confers long-lasting systemic disease resistance against a broad spectrum of pathogens and is associated with the expression of a certain set of genes (Ward et al. "Coordinate Gene Activity in Response to Agents that Induce Systemic Acquired Resistance," Plant Cell 3:1085-94 (1991)). SAR is an important component of the disease resistance of plants and has long been of interest, because the potential of inducing the plant to protect itself could significantly reduce or eliminate the need for chemical pesticides. SAR can be induced by biotic (microbes) and abiotic (chemical) agents (Gorlach et al. "Benzothiadiazole, a Novel Class of Inducers of Systemic Acquired Resistance, Activates Gene Expression and Disease Resistance in Wheat," Plant Cell 8:629-43 (1996)). Historically, weak virulent pathogens were used as a biotic inducing agent for SAR. Non-virulent plant growth promotion bacteria (PGPR) were also reported to be able to induce resistance of some plants against various diseases. Biotic agent-induced SAR has been the subject of much research, especially in the late 70s and early 80s. Only very limited success was achieved, however, due to: 1) inconsistency

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of the performance of living organisms in different environmental conditions;

2) considerable concerns regarding the unpredictable consequences of the intentional introduction of weakly virulent pathogens into the environment; and 3) the technical complication of applying a living microorganism into a variety of environmental

5 conditions. To overcome the limitations of using living organisms to induce SAR, scientists have long been looking for an HR elicitor derived from a pathogen for SAR induction. With the advancement of molecular biology, the first proteinaceous HR elicitor with broad host spectrum was isolated in 1992 from *Erwinia amylovora*, a pathogenic bacterium causing fire blight in apple and pear. The HR elicitor was

10 named "harpin". It consists of 403 amino acids with a molecular weight about 40 kDa. The harpin protein is heat-stable and glycine-rich with no cysteine. The gene encoding the harpin protein is contained in a 1.3 kB DNA fragment located in the middle of the *hrp* gene cluster. Harpin is secreted into the extracellular space and is very sensitive to proteinase digestion. Since the first harpin was isolated from

15 *Erwinia amylovora*, several harpin or harpin-like proteins have been isolated from other major groups of plant pathogenic bacteria. In addition to the harpin of *Erwinia amylovora*, the following harpin or harpin-like proteins have been isolated and characterized: HrpN of *Erwinia chrysanthemi*, *Erwinia carotovora* (Wei et al. "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen

20 *Erwinia amylovora*," Science, 257:85 (1992)), and *Erwinia stewartii*; HrpZ of *Pseudomonas syringae* (He et al. "Pseudomonas syringae pv. Syringae harpin_{pss}: A Protein that is Secreted Via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255 (1993)), PopA of *Ralstonia solanacearum*, (Arlat et al. "PopA1, a Protein which Induces a Hypersensitive-Like Response on Specific Petunia

25 Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-53 (1994)); HrpW of *Erwinia amylovora* (Kim et al., "HrpW of *Erwinia amylovora*, a New Harpin that Contains a Domain Homologous to Pectate Lyases of a Distinct Class," J. Bacteriol. 180:5203-10 (1998)), and *Pseudomonas syringae*. All of the currently described harpin or harpin-like proteins share common characteristics.

30 They are heat-stable and glycine-rich proteins with no cysteine amino acid residue, are very sensitive to digestion by proteinases, and elicit the HR and induce resistance in many plants against many diseases. Based on their shared biochemical and

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biophysical characteristics as well as biological functions, these HR elicitors from different pathogenic bacteria belong to a new protein family – i.e. the harpin protein family. The described characteristics, especially their ability to induce HR in a broad range of plants, distinguish the harpin protein family from other host specific
5 proteinaceous HR elicitors, for example elicitors from *Phytophthora* spp (Bonnet et al., “Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants,” Eur. J. Plant Path. 102:181-92 (1996); Keller, et al. “Physiological and Molecular Characteristics of Elicitor-Induced Systemic Acquired Resistance in Tobacco,” Plant Physiol 110:365-76 (1996)) or avirulence proteins (such as Avr9) from *Cladosporium*
10 *fulvum*, which are only able to elicit the HR in a specific variety or species of a plant.

In nature, when certain bacterial infections occur, harpin protein is expressed and then secreted by the bacteria, signaling the plant to mount a defense against the infection. Harpin serves as a signal to activate plant defense and other physiological systems, which include SAR, growth enhancement, and resistance to
15 certain insect damage.

The current understanding of critical plant molecules that may have a significant role in interacting with elicitors and then triggering a sequential signal transduction cascade is described as follows.

20 **Interaction of Plant Resistance Genes (R) and Pathogen Avirulence Genes (avr)**

The concept of gene-for-gene interaction is that “for each gene determining resistance (R gene) in the host, there is a corresponding gene determining avirulence in the pathogen (avr gene)”. In this model, pathogen avirulence genes
25 generate a specific ligand molecule, called an elicitor. Only plants carrying the matching resistance gene respond to this elicitor and invoke the HR. In the past few years, several disease-resistance, R genes, have been cloned and sequenced. It was expected that R genes might encode components involved in signal recognition or signal transduction pathways that ultimately lead to defense responses. The cloned R
30 genes could be grouped into four classes: (1) cytoplasmic protein kinase; (2) protein kinases with an extracellular domain; (3) cytoplasmic proteins with a region of leucine-rich repeats and a nucleotide-binding site; and (4) proteins with a region of

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leucine-rich repeats that appear to encode extracellular proteins. (Review in Bent, A.F. "Plant Disease Resistance Genes: Function Meets Structure," Plant Cell 8:1757-71 (1996); Baker B., et al., "Signaling in Plant-Microbe Interactions," Science 276:726-33 (1997)). The first R gene cloned, Pto, encodes a serine/threonine protein kinase. The protein product of Pto directly interacts with the cognate avirulence gene protein, AvrPro, which has been demonstrated in a yeast two-hybrid system. It was shown that only co-existence of both AvrPro and Pto proteins could elicit HR in plants (Tang et al., "Initiation of Plant Disease Resistance by Physical Interaction of AvrPto and Pto kinase," Science 274:2060-63 (1996); Scofield et al., "Molecular Basis of Gene-for-Gene Specificity in Bacterial Speck Disease of Tomato," Science 274:2063-65 (1996); Zhou et al., "The Pto kinase Conferring Resistance to Tomato Bacterial Speck Disease Interacts with Proteins that Bind a cis-element of Pathogenesis-related Genes," EMBO J. 16:3207-18 (1997)). The results from cloned R genes support the view that plant-pathogen interactions involve protein-protein interactions. Syringolide, a water-soluble, low-molecular-weight elicitor, triggers a defense response in soybean cultivars carrying the Rpg4 disease-resistance gene. A 34-KDa protein has been isolated from soybean and is considered to be the physiological active syringolide receptor (Ji et al., "Characterization of a 34-kDa Soybean Binding Protein for the syringolide Elicitors," Proc. Natl. Acad. Sci. USA 95:3306-11 (1998)).

Putative Binding Factor of Elicitin

Elicitins are a family of small proteins secreted by *Phytophthora* species that have a high degree of homology. Pure elicittins alone can cause a hypersensitive response, a local cell death, and trigger systemic acquired resistance in tobacco and other plants (Bonnet et al., "Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants," Eur. J. Plant Pathol. 102:181-92 (1996); Keller, et al. "Physiological and Molecular Characteristics of Elicitin-Induced Systemic Acquired Resistance in Tobacco," Plant Physiol 110:365-76 (1996)). However, the spectrum of HR elicitation and induced systemic resistance in plants is much narrower than that achieved by harpin family elicitors. Like harpin, elicittins induce a series of metabolic events in tobacco cells, including the accumulation of phytoalexins, ethylene

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production, transmembrane electrolyte leakage, H₂O₂ accumulation, and expression of plant defense related genes (Yu L, et al., "Elicitins from *Phytophthora* and Basic Resistance in Tobacco," Proc. Natl. (1995); Keller et al., "Pathogen-Induced Elicitin Production in Transgenic Tobacco Generates a Hypersensitive Response and

5 Nonspecific Disease Resistance," The Plant Cell 11:223-35 (1999)). A putative receptor-like binding factor has been identified in tobacco plasma membrane, which has a specific high-affinity to the cryptogein, one member of the elicitin family (Wendehenne, et al., "Evidence for Specific, High-Affinity Binding Sites for a Proteinaceous Elicitor in Tobacco Plasma Membrane," FEBS Letters 374:203-207

10 (1995)). Recently, it was found that 2 basic elicitors (i.e. cryptogein and cinnamomin) and two acidic elicitors (i.e. capsicein and parasitein) were able to interact with the same binding sites on tobacco plasma membranes (Bourque et al., "Comparison of Binding Properties and Early Biological Effects of Elicitins in Tobacco Cells," Plant Physiol. 118:1317-26 (1998)). However, the gene of the receptor-like factor has not

15 been isolated.

Putative Binding Factor of Glycoprotein Elicitors

A 42 kDa glycoprotein elicitor has been isolated from *Phytophthora*

20 *megasperma* (Parker et al., "An Extracellular Glycoprotein from *Phytophthora megasperma* f. sp. *glycinea* Elicits Phytoalexin Synthesis in Cultured Parsley Cells and Protoplasts," Mol. Plant Microbe Interact. 4:19-27 (1991)). An oligopeptide of 13 amino acids within the glycoprotein ("Pep-13") was able to induce a response in plants like that achieved by the full glycoprotein. A high affinity-binding pattern has

25 been observed in parsley microsomal membranes with an isotope labeled oligopeptide. There are estimated to be about 1600 to 2900 binding sites per cell with evidence indicating that a low abundant protein receptor of the Pep-13 is localized in the plasma membrane (Nurnberger et al., "High Affinity Binding of a Fungal Oligopeptide Elicitor to Parsley Plasma Membranes Triggers Multiple Defense

30 Responses," Cell 78:449-60 (1994)).

Harpin Protein Binding Factors

Harpin proteins, which elicit HR in a variety of different nonhost plants, have been isolated from plant pathogens (Wei et al. "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85 (1992)). A family of harpin proteins has been identified from plant bacterial pathogens. All of them have similar biological activities. It is well documented that harpin protein can induce plants to produce active oxygen, change ion flux, lead to local cell death, and induce systemic acquired resistance ("SAR") (Wei et al. "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85 (1992); He et al., "*Pseudomonas syringae* pv. *syringae* Harpin_{pss}: A Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-66 (1993); Baker, C.J., et al., "Harpin, an Elicitor of the Hypersensitive Response in Tobacco Caused by *Erwinia amylovora*, Elicits Active Oxygen Production in Suspension Cells," Plant Physiol. 102:1341-44 (1993)). No harpin protein binding factor has been isolated so far. It was reported that an amphipathic protein, named HRAP, isolated from sweet pepper could dissociate harpin_{pss} in multimeric form (*hrpZ* from *Pseudomonas syringae*). The biological activity of the HRAP is believed to be its ability to intensify harpin_{pss}-mediated hypersensitive response. HRAP protein does not bind to harpin_{pss} directly (Chen et al., "An Amphipathic Protein from Sweet Pepper can Dissociate Harpin_{pss} Multimeric Forms and Intensify the Harpin_{pss}-Mediated Hypersensitive Response," Physiological & Molecular Pathology 52:139-49 (1998)). Using a fluorochrome tagged antibody to harpin to examine the interaction of harpin_{pss} and tobacco suspension cells, it was found that harpin_{pss} interacted with the cultured cells, but not with protoplasts with the cell walls being digested and removed. It was interpreted that harpin_{pss} was localized in the outer portion of the plant cell, probably on the cell wall. However, it was not ruled out that the binding factor was located on the plasma membrane.

The present invention seeks to identify receptors for hypersensitive response elicitor proteins or polypeptides and uses of such receptors.

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SUMMARY OF THE INVENTION

The present invention is directed to an isolated protein which serves as a receptor in plants for a plant pathogen hypersensitive response elicitor. Also
5 disclosed are nucleic acid molecules encoding such receptors as well as expression vectors, host cells, transgenic plants, and transgenic plant seeds containing such nucleic acid molecules.

The protein of the present invention can be used with a method of identifying agents targeting plant cells by forming a reaction mixture including the
10 protein and a candidate agent, evaluating the reaction mixture for binding between the protein and the candidate agent, and identifying candidate compounds which bind to the protein in the reaction mixture as plant cell targeting agents.

The nucleic acid molecule of the present invention can be used in a method of identifying agents targeting plant cells by forming a reaction mixture
15 including a cell transformed with the nucleic acid molecule of the present invention and a candidate agent, evaluating the reaction mixture for binding between protein produced by the host cell and candidate agent, and identifying candidate compounds which bind to the protein or the host cell in the reaction mixture as plant cell targeting agents.

20 Another aspect of the present invention relates to a method of enhancing a plant's receptivity to treatment with hypersensitive response elicitors by providing a transgenic plant or transgenic plant seed transformed with the nucleic acid molecule of the present invention.

The present invention is also directed to a method of imparting disease
25 resistance, enhancing growth, controlling insects, and/or imparting stress resistance to plants by providing a transgenic plant or transgenic plant seed transformed with a DNA construct effective to silence expression of a nucleic acid molecule encoding a receptor in accordance with the present invention.

The discovery of the present invention has great significance. This
30 putative receptor protein can be used as a novel way to screen for new inducers of plant resistance against insect, disease, and stress, and of growth enhancement. This protein is the first step toward the understanding of the harpin induced signal transduction pathway in plants. Further studies of this pathway will provide more

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possible targets for new plant vaccine and growth enhancement products development. In addition, this protein can serve as an anchor providing a new way to target anything to the plant cells.

5

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a yeast two-hybrid screening with the *Erwinia amylovora* hypersensitive response elicitor (i.e. harpin) and a schematic representation of the interaction between harpin and a cDNA encoded polypeptide.

10 Harpin is fused to LexA protein which contains a DNA binding domain ("BD"). The cDNA encoded polypeptide is fused to the GAL4 transcription activation domain ("AD"). This interaction targets the activation domain to two different LexA-dependent promoters with consequent activation of the transcription of the *HIS3* and *lacZ* reporter genes.

15

Figures 2A-B show that the *Erwinia amylovora* hypersensitive response elicitor (i.e. harpin) is a good yeast two-hybrid bait. Reporter genes were not expressed in yeast strain L40 containing plasmids expressing the LexA - harpin fusion in combination with plasmids expressing the GAL4 activation domain alone, or fused to unrelated protein. Therefore, harpin is not autoactive in this yeast two-hybrid system. In addition, reporter genes were not expressed in yeast strain L40 containing plasmids expressing the GAL4 activation domain-harpin fusion in combination with plasmids expressing LexA alone, or fused to unrelated protein. Figure 2A shows a β -galactosidase assay where blue color indicates the expression of *lacZ* reporter gene. Figure 2B shows a synthetic minimal ("SD") media plate which lacks leucine, tryptophan, and histidine. Growth on such a plate indicates the expression of the *HIS3* reporter gene.

25

30

Figures 3A-B show the interaction between HrBP1 (hypersensitive response elicitor binding protein 1) and a hypersensitive response elicitor (i.e. harpin) is specific. Reporter genes were expressed in yeast strain L40 containing plasmids expressing the GAL4 activation domain-HrBP1 fusion in combination with plasmids expressing LexA fused to hypersensitive response elicitor (i.e. harpin), but were not expressed in combination with LexA alone, or LexA fused to unrelated proteins.

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Figure 3A is a β -galactosidase assay where the blue color indicates the expression of *lacZ* reporter gene. Figure 3B is an SD media plate which lacks leucine, tryptophan, and histidine. Growth on such a plate indicates the expression of the *HIS3* reporter gene.

5 Figures 4A-B show the interaction of HrBP1 and a hypersensitive response elicitor (i.e. harpin) in another orientation. Reporter genes were expressed in yeast strain L40 containing plasmids expressing the LexA - HrBP1 fusion in combination with plasmids expressing GAL4 activation domain fused to harpin, but were not expressed in combination with GAL4 activation domain alone, or GAL4
10 activation domain fused to unrelated proteins. Therefore, interaction between harpin and HrBP1 is specific. Figure 4A shows a β -galactosidase assay where blue color indicates the expression of *lacZ* reporter gene. Figure 4B shows an SD media plate which lacks leucine, tryptophan, and histidine. Growth on such a plate indicates the expression of the *HIS3* reporter gene.

15 Figure 5 shows the gene structure of HrBP1 and a schematic representation of the exons and introns of the HrBP1 gene. When comparing the HrBP1 cDNA sequence with the *Arabidopsis thaliana* genomic DNA sequence published in a public database, four exons and three introns were discovered.

20 Figure 6 shows a Northern blot using RNA probe complementary to bases 651-855 of HrBP1 coding region (SEQ. ID. No. 9).

 Figures 7A-B show that the interaction between rHrBP1 (R6) and harpin is specific. Reporter genes were expressed in yeast strain L40 containing plasmids expressing the GAL4 activation domain-rHrBP1 fusion in combination with plasmids expressing LexA fused to harpin or harpin 137-180 amino acids, but were
25 not expressed in combination with LexA alone, LexA fused to unrelated proteins, or fused to harpin 210-403 amino acids. Figure 7A shows a β -galactosidase assay where blue color indicates the expression of *lacZ* reporter gene. Figure 7B shows a SD media plate, which lacks leucine, tryptophan, and histidine. Growth on such a plate indicates the expression of the *HIS3* reporter gene.

30 Figure 8 shows the constructs used to "knockout" HrBP1 gene in *Arabidopsis*.

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Figure 9A-C show a *Pseudomonas syringae* p.v. tomato DC3000 assay on wild type and HrBP1 "knockout" transgenic Arabidopsis plants. Figure 9A is a picture taken 7 days after *P. syringae* inoculation. In Figure 9B, leaf disks were harvested. Bacteria were extracted from leaf disks and plated onto King's B agar plate containing 100µg/ml rifampicin. Figure 9C shows the bacteria count from plates in Figure 9B. This signifies an anti-sense line and d refers to a double-stranded RNA line.

Figure 10 shows the construct used to overexpress HrBP1 in tobacco.

Figure 11A-B show the height of wild type and HrBP1 overexpressing tobacco plants 52 days after they were transferred to soil. Figure 11A is a picture taken 52 days after plants were transferred to soil. Figure 11B shows average height of 8 plants per line.

Figure 12A-B show a TMV assay results on wild type and HrBP1 overexpressing tobacco plants. Figure 12A is a picture taken 3 days after TMV inoculation. Figure 12B shows the average virus lesion diameter from 5 plants per line 3 days after TMV inoculation.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to isolated receptors for hypersensitive response elicitor proteins or polypeptides. Also disclosed are DNA molecules encoding such receptors as well as expression systems, host cells, and plants containing such molecules. Uses of the receptors themselves and the DNA molecules encoding them are disclosed. The receptor for a hypersensitive response elicitor from a plant pathogen can be from a monocot or a dicot.

One example of such a receptor is that found in *Arabidopsis thaliana* which has the amino acid sequence of SEQ. ID. No. 1 as follows:

30	Met	Ala	Thr	Ser	Ser	Thr	Phe	Ser	Ser	Leu	Leu	Pro	Ser	Pro	Pro	Ala	1	5	10	15
	Leu	Leu	Ser	Asp	His	Arg	Ser	Pro	Pro	Pro	Ser	Ile	Arg	Tyr	Ser	Ph	20	25	30	
35	Ser	Pro	Leu	Thr	Thr	Pro	Lys	Ser	Ser	Arg	Leu	Gly	Phe	Thr	Val	Pro	35	40	45	

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Glu Lys Arg Asn Leu Ala Ala Asn Ser Ser Leu Val Glu Val Ser Ile
 50 55 60
 5 Gly Gly Glu Ser Asp Pro Pro Pro Ser Ser Ser Gly Ser Gly Gly Asp
 65 70 75 80
 Asp Lys Gln Ile Ala Leu Leu Lys Leu Lys Leu Leu Ser Val Val Ser
 85 90 95
 10 Gly Leu Asn Arg Gly Leu Val Ala Ser Val Asp Asp Leu Glu Arg Ala
 100 105 110
 Glu Val Ala Ala Lys Glu Leu Glu Thr Ala Gly Gly Pro Val Asp Leu
 115 120 125
 15 Thr Asp Asp Leu Asp Lys Leu Gln Gly Lys Trp Arg Leu Leu Tyr Ser
 130 135 140
 Ser Ala Phe Ser Ser Arg Ser Leu Gly Gly Ser Arg Pro Gly Leu Pro
 145 150 155 160
 Thr Gly Arg Leu Ile Pro Val Thr Leu Gly Gln Val Phe Gln Arg Ile
 165 170 175
 25 Asp Val Phe Ser Lys Asp Phe Asp Asn Ile Ala Glu Val Glu Leu Gly
 180 185 190
 Ala Pro Trp Pro Phe Pro Pro Leu Glu Ala Thr Ala Thr Leu Ala His
 195 200 205
 30 Lys Phe Glu Leu Leu Gly Thr Cys Lys Ile Lys Ile Thr Phe Glu Lys
 210 215 220
 Thr Thr Val Lys Thr Ser Gly Asn Leu Ser Gln Ile Pro Pro Phe Asp
 225 230 235 240
 Ile Pro Arg Leu Pro Asp Ser Phe Arg Pro Ser Ser Asn Pro Gly Thr
 245 250 255
 40 Gly Asp Phe Glu Val Thr Tyr Val Asp Asp Thr Met Arg Ile Thr Arg
 260 265 270
 Gly Asp Arg Gly Glu Leu Arg Val Phe Val Ile Ala
 275 280
 45

This protein, known as HrBP1p, is encoded by a cDNA molecule having SEQ. ID.

No. 2 as follows:

tttttccttc tcaacaatgg cgacttcttc tactttctcg tcactactac cttcaccacc 60
 50 agctcttctt tccgaccacc gttctcctcc accatccatc agatactcct tttctccctt 120
 aactactcca aaatcgtctc gtttggttt cactgtaccg gagaagagaa acctcgtgc 180
 taattcgtct ctcgttgaag tatccattgg cggagaaagt gaccaccac catcatcatc 240
 tggatcagga ggagacgaca agcaaattgc attactcaa ctcaaattac ttagtgtagt 300
 ttcgggatta aacagaggac ttgtggcgag tggtgatgat ttagaaagag ctgaagtggc 360
 55 tgctaaagaa cttgaaactg ctgggggacc gggtgattta accgatgac ttgataagct 420
 tcaagggaaa tggaggctgt tgtatagtag tgcgttctct tctcggctct taggtgtag 480
 ccgtcctggt ctacctactg gacgtttgat ccctgttact cttggccagg tgtttcaacg 540

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gattgatgtg tttagcaaag attttgataa catagcagag gtggaattag gagccccctg 600
 gccatttccg ccattagaag ccactgcgac attggcacac aagtttgaac tcttaggcac 660
 ttgcaagatc aagataacat ttgagaaaac aactgtgaag acatcgggaa acttgtcgca 720
 gattcctccg tttgatatcc cgaggcttcc cgacagtttc agaccatcgt caaaccttg 780
 5 aactggggat ttcgaagtta cctatgttga tgataccatg cgcataactc gcggggacag 840
 aggtgaactt aggggtattcg tcattgctta attctcaaag ctttgacatg taaagataaa 900
 taaatacttt ctgcttgatg cagtctcatg agttttgtac aaatcatgtg aacatataaa 960
 tgcgctttat aagtaaatga gtgtcttggt caatgaatca 1000

10 The genomic DNA molecule containing the receptor encoding cDNA
 molecule of SEQ. ID. No. 2 has SEQ. ID. No. 3 as follows:

aattagaaaa attaacaacc aacatctagt tagaatatth aatttgcacc aatgtcttcg 60
 agtatagtga aaaaaataga agatcgaata tcgaatagta cgtatagaat catctagatc 120
 15 cattcgaact aacgtctact tttcttttcc agcattaaca tgtagcttgt cattagcatt 180
 tacatgttgc aaataacaca aattgggaaa ttgaaagact aaaaaacctt gtacagcaga 240
 tggtttaaca cgtggattca tggacacaaa cagaaaacgg cagaactaag cacaaaaacg 300
 tcaactaagc atatcaaagc ttttaatgca agcctaatat aaacacaagt ggttatccat 360
 aatctgttct taatctcttg cagtagttat cttttcatta ttcgcaattc gcaattctat 420
 20 attcttatat ttcaacttgt tcttcttcca aattgtaatt atatctacat cgtcttagct 480
 tgaccattat agctccagta ccaagttctc ttcttaactt taatatcagc tactattctc 540
 atactgtaaa tatcttttgt tcaccaaaca tatatttcga accaaactgc taaaagctta 600
 tcataaattg cagttctagc cacacaattt tgcagttcca accattaaat gccacaaaat 660
 ttggacgatt tcttaagaca agaagaacat agcaaccaa ccttattgat taaatatgaa 720
 25 atgtctccat aaaactggga gatttcccca aataaagaga acacggcaaa tgttcacgta 780
 atctccaaga tgaatgttta attttttctt tcagaaaaaa acaaaaaaac ttaactcaat 840
 atagacaact agaattgata ccaactaagc aaaagaaatt caaaagacaa atatatatg 900
 gatatgaagt tacattatth tcaaacttta tatactacta aaagcctaaa aatttgttct 960
 aaaatgatat ccaataaat ggaaggcatg aatgtcatat gactaaaaga gaaaaacaca 1020
 30 cctgtatata agtattggat catgctgcct ccgagtgaaca aaacatacga tgtgggtctt 1080
 tattgggcca tacttaaatg gaaaaaggag aaaaaaatt gggcaatgtc tatggtcgaa 1140
 atttatatgt ttacatcaa taaaatcaat atttaattth atatatgtgg gtcttaactt 1200
 agtattatct acatagatta aaatcaaagt actgcatatg gtccataata atacaaccaa 1260
 agcaaattaa aattttgtgg cacaaaacga catcatthta ctcagaaagt aatatgcaat 1320
 35 ttcgtttacg cacacacgta tacgcgctaa taaccctggtg tgcttctcaa atcacataat 1380
 aattaaagtc ttcttcttct tcttcttctc tacaaattat ctcactctct tcgttttttt 1440
 ttccttctca acaatggcga cttcttctac tttctcgtca ctactacctt caccaccagc 1500
 tcttctttcc gaccaccgtt ctctctccacc atccatcaga tactcctttt ctcccttaac 1560
 tactccaaaa tcgtctcgtt tgggtttcac tgtaccggag aagagaaacc tcgctgctaa 1620
 40 ttcgtctctc gttgaagtat ccattggcgg agaaagtac ccaccacat catcatctgg 1680
 atcaggagga gacgacaagc aaattgcatt actcaaactc aaattacttg tgagtctgat 1740
 tcaaaccaat cggtgaaatt ataagaaatt gggttcgtt ctttggaatt aggggtttata 1800
 ttactgttaa gattcgatta tagagtgaat tttgggaaga ttttccagat ttgatttgtg 1860

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atgtgttgtg ttgtgagaaa ttgcagagtg tagtttcggg attaaacaga ggacttgtgg 1920
 cgagtgttga tgatttagaa agagctgaag tggctgctaa agaacttgaa actgctgggg 1980
 gaccggttga ttaaccgat gatcttgata agcttcaagg gaaatggagg ctgttgtata 2040
 gtagtgcggt ctcttctcgg tcttttaggtg gtagccgtcc tggctctacct actggacgtt 2100
 5 tgatccctgt tactcttggc caggtaattc ttgaatcatt gctctgtttt taccctgcaa 2160
 gattcgggtt ttcgggtaag ttgttgagga gtttatgtgc atggtctagt ctatcatcaa 2220
 tagtcttgct tgagtttgaa tggggctgag ctaagaatct agctttctga gggtacaatt 2280
 tggtaatgtc atcttatact cgaaagcaaa cttttttcta tattgtcaag tttatgtgta 2340
 cggctcgtgc tatcattggg agtctttgtt gagcttgaat ggtgaggagc ttagaatcta 2400
 10 gcaatgtcat ctactcctta atcatttttt tctatattgc caagtttatg tgtacggctc 2460
 tagtcaatca tctttattct tgggtgagtt tgaatggtga tgagcttaga atctagcttt 2520
 ctttggttta aatttggaag agaaccatac ctgaatcggg agaaagcaaa cttctttaat 2580
 attatctctt gtttctgaat cattaataca ggtgtttcaa cggattgatg tgtttagcaa 2640
 agattttgat aacatagcag aggtggaatt aggagcccct tggccatttc cgccattaga 2700
 15 agccactgcg acattggcac acaagtttga actcttaggt ttgcatthcc ctttctctca 2760
 ttaagtttat cgaattgtgt aagagcaaaa taacttatct gtatcttga catthtatggg 2820
 gaaaacaggc acttgcaaga tcaagataac atttgagaaa acaactgtga agacatcggg 2880
 aaacttgctg cagattcctc cgtttgatat cccgaggctt cccgacagtt tcagaccatc 2940
 gtcaaacctt ggaactgggg atttcgaagt tacctatgtt gatgatacca tgcgcataac 3000
 20 tcgcggggac agaggtgaac ttaggttatt cgtcattgct taattctcaa agctttgaca 3060
 tgtaagata aataaatact ttctgcttga tgcagtctca tgagttttgt acaaatcatg 3120
 tgaacatata aatgcgcttt ataagtaaat gagtgtcttg ttcaatgaat catatgaaag 3180
 aatttgatg actcagaaaa ttggacaatg atatagacct tccaaatttt gcaccctcta 3240
 atgtgagata ttagtgattt tttcttaggt tggtagagag aacggattgg caaaaaata 3300
 25 tcgaagggtc atgattaaca gcaaaacct atcttgatga ttcaaatat agagttaaca 3360
 agcaagatg agacaatctt atacgagaga gctaaaacaa atggattcca aatccagcaa 3420
 gtacaaaaat cgcagaaaat aagatgaac caacttaaaa cagagatgtt ccctttccct 3480
 tcttgctacc accgatctcg aaatgcttgc acctctgaaa taaacaacaa accaacacaa 3540
 tgtaagcaaa ttaccaagtt acaaatccgg tataatgaac tgatctatgt tctatgcacc 3600
 30 ttgataggac gctgcgaaaa gtgcttgacg ctttgacact gaagcctcaa aacaatcttc 3660
 ttcgtggtct tagcctgtta acaagattca caagatgtat ctcagtccaa aactgagact 3720
 attggaatgt ctgtttcctc acagctcact tccaaaattc tactataaat gggtccttaa 3780
 aactacctca tttcaactaa ctagacctaa ttcaaactga aaaaacaatc aatgcatgat 3840
 aatcaatgtt acctttttgt ggaagacagg cttagtctga ccaccataac cagattgttt 3900
 35 acggtcataa cgacgctttc cttgagcagc aagactgtct ttacccttct tgtattgggt 3960
 aacctgtgc aaagtatgct ttttgcatc cttgttctta cagtaagtgt tctttgtctt 4020
 tggaatgttc accttcaaaa ttcataaaat caaaaatgaa tcactcacac acatacaaaa 4080
 tcaagagact ttaaggtta atcaaaatac aaacatcatt tagattgaaa acttttatga 4140
 tagatctgaa aaacaatata ataaatcaat caaccatgta ttgttgttct tcaagtcaa 4200
 40 cgaactttac aaattccaaa atcacatcga aagagaagaa acaatttacc attttcgctg 4260

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Another example of a receptor in accordance with the present invention is that found in rice which has a partial amino acid sequence of SEQ. ID. No. 4 as follows:

```

5  Val Ala Ala Leu Lys Val Lys Leu Leu Ser Ala Val Ser Gly Leu Asn
    1           5           10           15
    Arg Gly Leu Ala Gly Ser Gln Glu Asp Leu Asp Arg Ala Asp Ala Ala
    20           25           30
10  Ala Arg Glu Leu Glu Ala Ala Ala Gly Gly Gly Pro Val Asp Leu Glu
    35           40           45
    Arg Asp Val Asp Lys Leu Gln Gly Arg Trp Arg Leu Val Tyr Ser Ser
    50           55           60
15  Ala Phe Ser Ser Arg Thr Leu Gly Gly Ser Arg Pro Gly Pro Pro Thr
    65           70           75           80
    Gly Arg Leu Leu Pro Ile Thr Leu Gly Gln Val Phe Gln Arg Ile Asp
    85           90           95
    Val Val Ser Lys Asp Phe Asp Asn Ile Val Asp Val Glu Leu Gly Ala
    100          105          110
25  Pro Trp Pro Leu Pro Pro Val Glu Leu Thr Ala Thr Leu Ala His Lys
    115          120          125
    Phe Glu Ile Ile Gly Thr Ser Ser Ile Lys Ile Thr Phe Asp Lys Thr
    130          135          140
    Thr Val Lys Thr Lys Gly Asn Leu Ser Gln Leu Pro Pro Leu Glu Val
    145          150          155          160
35  Pro Arg Ile Pro Asp Asn Leu Arg Pro Pro Ser Asn Thr Gly Ser Gly
    165          170          175
    Glu Phe Glu Val Thr Tyr Leu Asp Gly Asp Thr Arg Ile Thr Arg Gly
    180          185          190
40  Asp Arg Gly Glu Leu Arg Val Phe Val Ile Ser
    195          200

```

45 This protein, known as R6p, is encoded by a cDNA molecule which has a partial sequence corresponding to SEQ. ID. No. 5 as follows:

```

50  cgtggctgcg ctcaaagtca agcttctgag cgcggtgtcc gggetgaacc gcggcctcgc 60
    ggggagccag gaggatcttg accgcgccga cgcggcggcg cgggagctcg aggcggcggc 120
    ggggtggcggc ccgctcgacc tggagagggg cgtggacaag ctgcaggggc ggtggaggct 180
    ggtgtacagc agcgcgttct cgtcgcggac gtcggcggc agccgccccg gcccgccac 240
    cggccgcctc ctccccatca ccctcgggca ggtgtttcag aggatcgatg ttgtcagcaa 300
    ggacttcgac aacatcgctc atgtcgagct cggcgcgcca tggccgctgc cgccggtgga 360
55  gctgacggcg accctggctc acaagtttga gatcatcggc acctcgagca taaagatcac 420
    attcgacaag acgacggtga agacgaaggg gaacctgtcc cagctgccgc cgctggaggt 480
    ccctcgcatc ccggacaacc tccggccgcc gtccaacacc ggcagcggcg agttcgaggt 540
    gacctacctc gacggcgaca cccgcatcac ccgcggggac agagggggagc tcagggtgtt 600

```

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cgatcatctcg tga

613

Hypersensitive response elicitors recognized by the receptors of the present invention are able to elicit local necrosis in plant tissue contacted by the elicitor.

Examples of suitable bacterial sources of hypersensitive response elicitor polypeptides or proteins include *Erwinia*, *Pseudomonas*, and *Xanthomonas* species (e.g., the following bacteria: *Erwinia amylovora*, *Erwinia chrysanthemi*, *Erwinia stewartii*, *Erwinia carotovora*, *Pseudomonas syringae*, *Pseudomonas solanacearum*, *Xanthomonas campestris*, and mixtures thereof).

An example of a fungal source of a hypersensitive response elicitor protein or polypeptide is *Phytophthora*. Suitable species of *Phytophthora* include *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamomi*, *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora*.

The hypersensitive response elicitor polypeptide or protein from *Erwinia chrysanthemi* is disclosed in U.S. Patent No. 5,850,015 and U.S. Patent No. 6,001,959, which are hereby incorporated by reference. This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34 kDa, is heat stable, has a glycine content of greater than 16%, and contains substantially no cysteine.

The hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* has a molecular weight of about 39 kDa, has a pI of approximately 4.3, and is heat stable at 100°C for at least 10 minutes. This hypersensitive response elicitor polypeptide or protein has a glycine content of greater than 21% and contains substantially no cysteine. The hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* is more fully described in U.S. Patent No. 5,849,868 to Beer and Wei, Z.-M., et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which are hereby incorporated by reference.

The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas syringae* has a molecular weight of 34-35 kDa. It is rich in glycine (about 13.5%) and lacks cysteine and tyrosine. Further information about the hypersensitive response elicitor derived from *Pseudomonas syringae* and its encoding

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DNA molecule is found in U.S. Patent Nos. 5,708,139 and 5,858,786 and He et al., "Pseudomonas syringae pv. syringae Harpin_{PS}: A Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-66 (1993), which are hereby incorporated by reference.

5 The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* is set forth in Arlat, M., F. Van Gijsegem, J. C. Huet, J. C. Pemollet, and C. A. Boucher, "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-533 (1994), which is
10 hereby incorporated by reference. This protein has 344 amino acids, a molecular weight of 33.2 kDa, and a pI of 4.16, is heat stable and glycine rich (20.6%).

 The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* pv. *glycines* has a partial amino acid sequence corresponding to SEQ. ID. No. 6 as follows:

15 Thr Leu Ile Glu Leu Met Ile Val Val Ala Ile Ile Ala Ile Leu Ala
 1 5 10 15
20 Ala Ile Ala Leu Pro Ala Tyr Gln Asp Tyr
 20 25

 This sequence is an amino terminal sequence having only 26 residues from the hypersensitive response elicitor polypeptide or protein of *Xanthomonas campestris*
25 pv. *glycines*. It matches with fimbrial subunit proteins determined in other *Xanthomonas campestris* pathovars.

 The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* pv. *pelargonii* is heat stable, protease sensitive, and has a
30 molecular weight of 20 kDa. It has the amino acid sequence of SEQ. ID. No. 7 as follows:

 Met Asp Ser Ile Gly Asn Asn Phe Ser Asn Ile Gly Asn Leu Gln Thr
 1 5 10 15
35 Met Gly Ile Gly Pro Gln Gln His Glu Asp Ser Ser Gln Gln Ser Pro
 20 25 30
 Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln Leu Leu Ala Met Phe Ile
 35 40 45
40

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Met Met Met Leu Gln Gln Ser Gln Gly Ser Asp Ala Asn Gln Glu Cys
 50 55 60

5 Gly Asn Glu Gln Pro Gln Asn Gly Gln Gln Glu Gly Leu Ser Pro Leu
 65 70 75 80

Thr Gln Met Leu Met Gln Ile Val Met Gln Leu Met Gln Asn Gln Gly
 85 90 95

10 Gly Ala Gly Met Gly Gly Gly Gly Ser Val Asn Ser Ser Leu Gly Gly
 100 105 110

Asn Ala

15

This amino acid sequence is encoded by the nucleotide sequence of SEQ. ID. No. 8 as follows:

20 atggactcta tcggaacaa cttttcgaat atcggaacc tgcagacgat gggcatcggg 60
 cctcagcaac acgaggactc cagccagcag tcgccttcgg ctggctccga gcagcagctg 120
 gatcagttgc tcgccatgtt catcatgatg atgctgcaac agagccaggg cagcgatgca 180
 aatcaggagt gtggcaacga acaaccgcag aacggtcaac aggaaggcct gagtccgttg 240
 acgcagatgc tgatgcagat cgtgatgcag ctgatgcaga accagggcgg cgccggcatg 300
 25 ggcgggtggcg gttcgggtcaa cagcagcctg ggcggcaacg cc 342

Isolation of *Erwinia carotovora* hypersensitive response elicitor protein or polypeptide is described in Cui et al., "The RsmA Mutants of *Erwinia carotovora*
 30 subsp. *carotovora* Strain Ecc71 Overexpress *hrp* N_{Ecc} and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI, 9(7):565-73 (1996), which is hereby incorporated by reference. This protein has 356 amino acids, a molecular weight of 35.6 kDa, and a pI of 5.82 and is heat stable and glycine rich (21.3%).

The hypersensitive response elicitor protein or polypeptide of *Erwinia*
 35 *stewartii* is set forth in Ahmad et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microbe Interact., July 14-19, 1996 and Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc., July 27-31, 1996, which are hereby incorporated by reference.

40 Hypersensitive response elicitor proteins or polypeptides from *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamoni*, *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora* are

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described in Kaman, et al., "Extracellular Protein Elicitors from Phytophthora: Most Specificity and Induction of Resistance to Bacterial and Fungal Phytopathogens," Molec. Plant-Microbe Interact., 6(1):15-25 (1993), Ricci et al., "Structure and Activity of Proteins from Pathogenic Fungi Phytophthora Eliciting Necrosis and Acquired Resistance in Tobacco," Eur. J. Biochem., 183:555-63 (1989), Ricci et al., "Differential Production of Parasiticein, and Elicitor of Necrosis and Resistance in Tobacco, by Isolates of Phytophthora parasitica," Plant Path. 41:298-307 (1992), Baillreul et al, "A New Elicitor of the Hypersensitive Response in Tobacco: A Fungal Glycoprotein Elicits Cell Death, Expression of Defence Genes, Production of Salicylic Acid, and Induction of Systemic Acquired Resistance," Plant J., 8(4):551-60 (1995), and Bonnet et al., "Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants," Eur. J. Plant Path., 102:181-92 (1996), which are hereby incorporated by reference. These hypersensitive response elicitors from *Phytophthora* are called elicitins. All known elicitins have 98 amino acids and show >66% sequence identity. They can be classified into two groups, the basic elicitins and the acidic elicitins, based on the physicochemical properties. This classification also corresponds to differences in the elicitins' ability to elicit HR-like symptoms. Basic elicitins are 100 times more effective than the acidic ones in causing leaf necrosis on tobacco plants.

The hypersensitive response elicitor from Gram positive bacteria like *Clavibacter michiganensis* is described in WO 99/11133, which is hereby incorporated by reference.

The above elicitors are exemplary. Other elicitors can be identified by growing fungi or bacteria that elicit a hypersensitive response using conditions under which genes encoding an elicitor are expressed. Cell-free preparations from culture supernatants can be tested for elicitor activity (i.e. local necrosis) by using them to infiltrate appropriate plant tissues.

Turning again to the receptor of the present invention for such hypersensitive response elicitors, fragments of the above receptor protein are encompassed by the method of the present invention. In addition, fragments of full length receptor proteins from other plants can also be utilized.

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Suitable fragments can be produced by several means. In the first, subclones of the gene encoding a known receptor protein are produced by conventional molecular genetic manipulation by subcloning gene fragments. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or peptide that can be tested for receptor activity according to the procedure described above.

As an alternative, fragments of a receptor protein can be produced by digestion of a full-length receptor protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave receptor proteins at different sites based on the amino acid sequence of the receptor protein. Some of the fragments that result from proteolysis may be active receptors.

In another approach, based on knowledge of the primary structure of the receptor protein, fragments of the receptor protein gene may be synthesized by using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. These then would be cloned into an appropriate vector for expression of a truncated peptide or protein.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences for the receptor being produced. Alternatively, subjecting a full length receptor to high temperatures and pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

Variants may be made by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure, and hydrophathic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a tag or other sequence for ease of synthesis, purification, or identification of the polypeptide.

Suitable DNA molecules are those that hybridize to a DNA molecule comprising a nucleotide sequence of 50 continuous bases of SEQ. ID. No. 2 under stringent conditions characterized by a hybridization buffer comprising 0.9M sodium

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citrate ("SSC") buffer at a temperature of 37°C and remaining bound when subject to washing with the SSC buffer at 37°C; and preferably in a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C and remaining bound when subject to washing at 42°C with 0.2x SSC buffer at 42°C.

The receptor of the present invention is preferably produced in purified form (preferably at least about 60%, more preferably 80%, pure) by conventional techniques. Typically, the receptor of the present invention is produced but not secreted into the growth medium of recombinant host cells. Alternatively, the receptor protein of the present invention is secreted into growth medium. In the case of unsecreted protein, to isolate the receptor protein, the host cell (e.g., *E. coli*) carrying a recombinant plasmid is propagated, lysed by sonication, or chemical treatment, and the homogenate is centrifuged to remove bacterial debris. The cell lysate can be further purified by conventionally utilized chromatography procedures (e.g., gel filtration in an appropriately sized dextran or polyacrylamide column to separate the receptor protein). If necessary, the protein fraction may be further purified by ion exchange or HPLC.

The DNA molecule encoding the receptor protein can be incorporated in cells using conventional recombinant DNA technology. Generally, this involves inserting the DNA molecule into an expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including procaryotic organisms and eucaryotic cells grown in tissue culture.

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Recombinant genes may also be introduced into viruses, such as vaccinia virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited to, the following viral
5 vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which is hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes,"
10 Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard
15 cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference.

A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host
20 cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA; microorganisms such as yeast containing yeast vectors; mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression
25 elements of these vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Different genetic signals and processing events control many levels of gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation).

30 Transcription of DNA is dependent upon the presence of a promotor which is a DNA sequence that directs the binding of RNA polymerase and thereby promotes mRNA synthesis. The DNA sequences of eucaryotic promotors differ from

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those of procaryotic promotors. Furthermore, eucaryotic promotors and accompanying genetic signals may not be recognized in or may not function in a procaryotic system, and, further, procaryotic promotors are not recognized and do not function in eucaryotic cells.

5 Similarly, translation of mRNA in procaryotes depends upon the presence of the proper procaryotic signals which differ from those of eucaryotes. Efficient translation of mRNA in procaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG,
10 which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby
15 incorporated by reference.

 Promotors vary in their "strength" (i.e. their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promotors in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a
20 number of suitable promotors may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promotors such as the T7 phage promoter, *lac* promoter, *trp* promoter, *recA* promoter, ribosomal RNA promoter, the P_R and P_L promotors of coliphage lambda and others, including but not limited, to *lacUV5*, *ompF*, *bla*, *lpp*, and the like, may be used to direct high levels of transcription of adjacent DNA
25 segments. Additionally, a hybrid *trp-lacUV5 (tac)* promoter or other *E. coli* promotors produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

 Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promoter unless specifically induced. In certain operations,
30 the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG

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(isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

Specific initiation signals are also required for efficient gene transcription and translation in procaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promotor, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon ("ATG") to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

Once the isolated DNA molecule encoding the receptor protein has been cloned into an expression system, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like.

One aspect of the present invention involves enhancing a plant's receptivity to treatment with a hypersensitive response elicitor by providing a transgenic plant or transgenic plant seed, transformed with a nucleic acid molecule encoding a receptor protein for a hypersensitive response elicitor. It has been found that hypersensitive response elicitors are useful in imparting disease resistance to plants, enhancing plant growth, effecting insect control and/or imparting stress resistance in a variety of plants. In view of the receptor of the present invention's interaction with such elicitors, it is expected that these beneficial effects would be enhanced by carrying out such elicitor treatments with plants transformed with the receptor encoding gene of the present invention.

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Transgenic plants containing a gene encoding a receptor in accordance with the present invention can be prepared according to techniques well known in the art.

5 A vector containing the receptor encoding gene described above can be microinjected directly into plant cells by use of micropipettes to transfer mechanically the recombinant DNA. Crossway, Mol. Gen. Genetics, 202:179-85 (1985), which is hereby incorporated by reference. The genetic material may also be transferred into the plant cell using polyethylene glycol. Krens, et al., Nature, 296:72-74 (1982), which is hereby incorporated by reference.

10 Another approach to transforming plant cells with a gene is particle bombardment (also known as biolistic transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford et al., which are hereby
15 incorporated by reference. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be
20 surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells.

Yet another method of introduction is fusion of protoplasts with other entities, either minicells, cells, lysosomes, or other fusible lipid-surfaced bodies.
25 Fraley, et al., Proc. Natl. Acad. Sci. USA, 79:1859-63 (1982), which is hereby incorporated by reference.

The DNA molecule may also be introduced into the plant cells by electroporation. Fromm et al., Proc. Natl. Acad. Sci. USA, 82:5824 (1985), which is hereby incorporated by reference. In this technique, plant protoplasts are
30 electroporated in the presence of plasmids containing the expression cassette. Electrical impulses of high field strength reversibly permeabilize biomembranes

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allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate.

Another method of introducing the DNA molecule into plant cells is to infect a plant cell with *Agrobacterium tumefaciens* or *A. rhizogenes* previously transformed with the gene. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration medium without antibiotics at 25-28°C.

Agrobacterium is a representative genus of the gram-negative family Rhizobiaceae. Its species are responsible for crown gall (*A. tumefaciens*) and hairy root disease (*A. rhizogenes*). The plant cells in crown gall tumors and hairy roots are induced to produce amino acid derivatives known as opines, which are catabolized only by the bacteria. The bacterial genes responsible for expression of opines are a convenient source of control elements for chimeric expression cassettes. In addition, assaying for the presence of opines can be used to identify transformed tissue.

Heterologous genetic sequences can be introduced into appropriate plant cells, by means of the Ti plasmid of *A. tumefaciens* or the Ri plasmid of *A. rhizogenes*. The Ti or Ri plasmid is transmitted to plant cells on infection by Agrobacterium and is stably integrated into the plant genome. J. Schell, Science, 237:1176-83 (1987), which is hereby incorporated by reference.

After transformation, the transformed plant cells must be regenerated.

Plant regeneration from cultured protoplasts is described in Evans et al., Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants, Acad. Press, Orlando, Vol. I, 1984, and Vol. III (1986), which are hereby incorporated by reference.

It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to, all major species of sugarcane, sugar beets, cotton, fruit trees, and legumes.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing

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transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

10 After the expression cassette is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

 Once transgenic plants of this type are produced, the plants themselves can be cultivated in accordance with conventional procedures. Alternatively, transgenic seeds or propagules (e.g., cuttings) are recovered from the transgenic plants. The seeds can then be planted in the soil and cultivated using conventional procedures to produce transgenic plants. The transgenic plants are propagated from the planted transgenic seeds.

 These elicitor treatment methods can involve applying the hypersensitive response elicitor polypeptide or protein in a non-infectious form to all or part of a plant or a plant seed transformed with a receptor gene in accordance with the present invention under conditions effective for the elicitor to impart disease resistance, enhance growth, control insects, and/or to impart stress resistance. Alternatively, the hypersensitive response elicitor protein or polypeptide can be applied to plants such that seeds recovered from such plants themselves are able to impart disease resistance in plants, to enhance plant growth, to effect insect control, and/or to impart resistance to stress.

 As an alternative to applying a hypersensitive response elicitor polypeptide or protein to plants or plant seeds in order to impart disease resistance in plants, to effect plant growth, to control insects, and/or to impart stress resistance in the plants or plants grown from the seeds, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant

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transformed with both a DNA molecule encoding a receptor in accordance with the present invention and with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein. The plant is grown under conditions effective to permit the DNA molecules to impart disease resistance to plants, to enhance plant growth, to control insects, and/or to impart resistance to stress. Alternatively, a transgenic plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and a DNA molecule encoding a receptor can be provided and planted in soil. A plant is then propagated from the planted seed under conditions effective to permit the DNA molecules to impart disease resistance to plants, to enhance plant growth, to control insects, and/or to impart resistance to stress.

The embodiment where the hypersensitive response elicitor polypeptide or protein is applied to the plant or plant seed can be carried out in a number of ways, including: 1) application of an isolated elicitor or 2) application of bacteria which do not cause disease and are transformed with a gene encoding the elicitor. In the latter embodiment, the elicitor can be applied to plants or plant seeds by applying bacteria containing the DNA molecule encoding the hypersensitive response elicitor polypeptide or protein. Such bacteria must be capable of secreting or exporting the elicitor so that the elicitor can contact plant or plant seeds cells. In these embodiments, the elicitor is produced by the bacteria *in planta* or on seeds or just prior to introduction of the bacteria to the plants or plant seeds.

The hypersensitive response elicitor treatment can be utilized to treat a wide variety of plants or their seeds to impart disease resistance, enhance growth, control insects, and/or impart stress resistance. Suitable plants include dicots and monocots. More particularly, useful crop plants can include: alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane. Examples of suitable ornamental plants are: *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

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With regard to the use of hypersensitive response elicitors in imparting disease resistance, absolute immunity against infection may not be conferred, but the severity of the disease is reduced and symptom development is delayed. Lesion number, lesion size, and extent of sporulation of fungal pathogens are all decreased.

5 This method of imparting disease resistance has the potential for treating previously untreatable diseases, treating diseases systemically which might not be treated separately due to cost, and avoiding the use of infectious agents or environmentally harmful materials.

The method of imparting pathogen resistance to plants is useful in

10 imparting resistance to a wide variety of pathogens including viruses, bacteria, and fungi. Resistance, *inter alia*, to the following viruses can be achieved by the method of the present invention: *Tobacco mosaic virus* and *Tomato mosaic virus*. Resistance, *inter alia*, to the following bacteria can also be imparted to plants *Pseudomonas solanacearum*; *Pseudomonas syringae* pv. *tabaci*; and *Xanthomonas*

15 *campestris* pv. *pelargonii*. Plants can be made resistant, *inter alia*, to the following fungi: *Fusarium oxysporum* and *Phytophthora infestans*.

With regard to the use of the hypersensitive response elicitor protein or polypeptide to enhance plant growth, various forms of plant growth enhancement or promotion can be achieved. This can occur as early as when plant growth begins

20 from seeds or later in the life of a plant. For example, plant growth according to the present invention encompasses greater yield, increased quantity of seeds produced, increased percentage of seeds germinated, increased plant size, greater biomass, more and bigger fruit, earlier fruit coloration, and earlier fruit and plant maturation. As a result, there is significant economic benefit to growers. For example, early

25 germination and early maturation permit crops to be grown in areas where short growing seasons would otherwise preclude their growth in that locale. Increased percentage of seed germination results in improved crop stands and more efficient seed use. Greater yield, increased size, and enhanced biomass production allow greater revenue generation from a given plot of land.

30 The use of hypersensitive response elicitors for insect control encompasses preventing insects from contacting plants to which the hypersensitive response elicitor has been applied, preventing direct insect damage to plants by

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feeding injury, causing insects to depart from such plants, killing insects proximate to such plants, interfering with insect larval feeding on such plants, preventing insects from colonizing host plants, preventing colonizing insects from releasing phytotoxins, etc. The present invention also prevents subsequent disease damage to plants
5 resulting from insect infection.

Elicitor treatment is effective against a wide variety of insects.

European corn borer is a major pest of corn (dent and sweet corn) but also feeds on over 200 plant species including green, wax, and lima beans and edible soybeans, peppers, potato, and tomato plus many weed species. Additional insect larval feeding
10 pests which damage a wide variety of vegetable crops include the following: beet armyworm, cabbage looper, corn ear worm, fall armyworm, diamondback moth, cabbage root maggot, onion maggot, seed corn maggot, pickleworm (melonworm), pepper maggot, tomato pinworm, and maggots. Collectively, this group of insect pests represents the most economically important group of pests for vegetable
15 production worldwide.

Hypersensitive response elicitor treatment is also useful in imparting resistance to plants against environmental stress. Stress encompasses any environmental factor having an adverse effect on plant physiology and development. Examples of such environmental stress include climate-related stress (e.g., drought,
20 water, frost, cold temperature, high temperature, excessive light, and insufficient light), air pollution stress (e.g., carbon dioxide, carbon monoxide, sulfur dioxide, NO_x, hydrocarbons, ozone, ultraviolet radiation, acidic rain), chemical (e.g., insecticides, fungicides, herbicides, heavy metals), and nutritional stress (e.g., fertilizer, micronutrients, macronutrients).

25 The application of the hypersensitive response elicitor polypeptide or protein can be carried out through a variety of procedures when all or part of the plant is treated, including leaves, stems, roots, etc. This may (but need not) involve infiltration of the hypersensitive response elicitor polypeptide or protein into the plant. Suitable application methods include high or low pressure spraying, injection, and
30 leaf abrasion proximate to when elicitor application takes place. When treating plant seeds or propagules (e.g., cuttings), the hypersensitive response elicitor protein or polypeptide can be applied by low or high pressure spraying, coating, immersion, or

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injection. Other suitable application procedures can be envisioned by those skilled in the art provided they are able to effect contact of the elicitor with cells of the plant or plant seed. Once treated with a hypersensitive response elicitor, the seeds can be planted in natural or artificial soil and cultivated using conventional procedures to
5 produce plants. After plants have been propagated from seeds treated with an elicitor, the plants may be treated with one or more applications of the hypersensitive response elicitor protein or polypeptide to impart disease resistance to plants, to enhance plant growth, to control insects on the plants, and/or to impart stress resistance.

The hypersensitive response elicitor polypeptide or protein can be
10 applied to plants or plant seeds alone or in a mixture with other materials. Alternatively, the elicitor can be applied separately to plants with other materials being applied at different times.

A composition suitable for treating plants or plant seeds contains a hypersensitive response elicitor polypeptide or protein in a carrier. Suitable carriers
15 include water, aqueous solutions, slurries, or dry powders.

Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, nematocide, and mixtures thereof. Suitable fertilizers include $(\text{NH}_4)_2\text{NO}_3$. An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

20 Other suitable additives include buffering agents, wetting agents, coating agents, and abrading agents. In addition, the hypersensitive response elicitor can be applied to plant seeds with other conventional seed formulation and treatment materials, including clays and polysaccharides.

In the alternative technique involving the use of transgenic plants and
25 transgenic seeds encoding a hypersensitive response elicitor encoding gene, a hypersensitive response elicitor need not be applied topically to the plants or seeds. Instead, transgenic plants transformed with a DNA molecule encoding such an elicitor are produced according to procedures well known in the art as described above.

In another embodiment, the present invention relates to a DNA
30 construct which is an antisense nucleic acid molecule to a nucleic acid molecule encoding a receptor in plants for plant pathogen hypersensitive response elicitors. An example of such a construct would be an antisense DNA molecule of the DNA

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molecule having the nucleotide sequence of SEQ. ID. Nos. 2 or 3. Alternatively, the DNA construct can have a DNA molecule having the nucleotide sequence of SEQ. ID. Nos. 2 or 3 (or a portion thereof) and its complementary strand and is used to generate a single transcript with an inverted repeat (i.e. a double-stranded) RNA. This transcript as well as the above-discussed antisense nucleic acid molecule can be used to induce silencing of a nucleic acid molecule encoding a receptor for a hypersensitive response elicitor.

Sensing the hypersensitive response elicitor by the receptor is the very first step of the signal transduction pathway in plants which eventually leads to disease resistance, growth enhancement, insect control, and stress resistance. Silencing the receptor provides a powerful tool to find and study the downstream components of this pathway. Additionally, the receptor could be a negative regulator of such plant signal transduction pathway. Silencing of the receptor will impart to plants the ability to resist disease and stress, control insects, and enhance growth without hypersensitive response elicitor treatment.

EXAMPLES

Example 1 - Materials and Methods

The laboratory technique used in the following example is straight forward. All DNA manipulations described here followed conventional protocols (Sambrook et al., "Molecular Cloning: A Laboratory Manual," 2nd ed., Cold Spring Harbor Laboratory (1989); Ausubel, et al., "Current Protocols in Molecular Biology," John Wiley (1987), which are hereby incorporated by reference). The plasmids and microorganisms described herein, used for making the present invention, were obtained from commercial sources, or from the authors of previous publications. Sequences were analyzed with Clone Manager 5 (Scientific & Educational Software, Durham, North Carolina).

Yeast strain L40 was grown in YPD or in different minimal synthetic dropout selection media at 30°C. *E.coli* strains DH5α and HB101 were grown in LB at 37°C.

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The yeast Two-Hybrid system is based on the fact that many eukaryotic transcription factors are composed of a physically separable, functionally independent DNA-binding domain (DNA-BD) and an activation domain (AD). Both the DNA-BD and the AD are required to activate a gene. When physically separated
5 by recombinant DNA technology and expressed in the same host cell, the DNA-BD and the AD do not interact directly with each other and, thus, cannot activate the responsive gene (Ma, et al., "Converting a Eukaryotic Transcriptional Inhibitor into an Activator," Cell 55:443 (1988) and Brent, et al., "A Eukaryotic Transcriptional Activator Bearing the DNA Specificity of a Prokaryotic Repressor," Cell 43:729
10 (1985), which are hereby incorporated by reference). But if the DNA-BD and the AD are brought into close physical proximity in the promoter region, the transcriptional activation function will be restored. Therefore, the yeast *Saccharomyces cerevisiae* and the Two-Hybrid system have become essential genetic tools for studying the macromolecular interactions.

15 In the Two-Hybrid system utilized here, the DNA-BD, encoded in the bait vector pVJL11 (Jullien-Flores, V., "Bridging Ral GTPase to Rho Pathways. RLIP76, a Ral Effector with CDC42/Rac GTPase-activating Protein Activity," J. Biol. Chem. 27:22473 (1995), which is hereby incorporated by reference), is the prokaryotic LexA protein, and the activation domain, encoded in the prey vector
20 pGAD 10 or pGAD GH (Clontech; Hannon, GJ., "Isolation of the Rb-related p130 Through its Interaction with CDK2 and Cyclins," Genes Dev. 7:2378 (1993), which is hereby incorporated by reference) is derived from the yeast GAL4 protein. pVJL11 also has a *TRP1* marker, and the pGAD a *LEU2* marker. An interaction between the bait protein (fused to the DNA-BD) and a library-encoded protein (fused to the AD)
25 creates a novel transcriptional activator with binding affinity for LexA operators. The yeast host L40 {MATa his3D200 trp1-901 leu2-3, 112 ade2 LYS2::(lexAop)₄-HIS3 URA3::(lexAop)₈-lacZ} harbors two reporter genes, *lacZ* and *HIS3*, which contain upstream LexA binding site. The *HIS3* nutritional reporter provides a sensitive growth selection that can identify a single positive transformant out of several million
30 candidate clones. The expression of the reporter genes indicates interaction between a candidate protein and the bait protein. See Figure 1.

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Erwinia amylovora harpin was used as the bait protein to screen the *Arabidopsis thaliana* MATCHMAKER cDNA library cloned in the pGAD 10 vector (Clontech Laboratories, Inc., Palo Alto, California). One cDNA library encoded protein was identified as a strong harpin interacting protein and, thus, a putative harpin receptor. The present invention reports the nucleic acid sequence and the deduced amino acid sequence of this cDNA.

Example 2

HrpN of *Erwinia amylovora* was subcloned into the yeast Two-Hybrid bait vector pVJL11. PCR was carried out using the 1.3 Kb harpin fragment (Wei et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85 (1992), which is hereby incorporated by reference) as a template to amplify the harpin encoding region. A Bam HI site was added to the 5' end of the coding sequence, and a Sal I site to the 3' end. A Bam HI and Sal I digested PCR fragment was ligated with the bait vector pVJL11 digested with the same restriction enzymes. pVJL11 has a *TRP1* marker to be selected in yeast and an Amp resistance marker to be selected in *E. coli*. The plasmid DNA was amplified in *E. coli* strain DH5 α . When tested in the Two-Hybrid system with empty prey vector pGAD GH and several unrelated proteins, HrpN didn't show auto-activation or nonspecific interaction with unrelated proteins, as shown in Figure 2.

Example 3

HrpN-pVJL 11 was transformed into yeast strain L40 by a lithium acetate (LiAc)-mediated method (Ito et al., "Transformation of Intact Yeast Cells Treated with Alkali Cations," J. Bacteriol. 153:163 (1983) and Vojtek et al., "Mammalian Ras Interacts Directly with the Serine/Threonine Kinase Raf.," Cell 74:205 (1993), which are hereby incorporated by reference). The *Arabidopsis thaliana* MATCHMAKER cDNA library (Clontech Laboratories, Inc., Palo Alto, CA) was screened for harpin interacting proteins. Approximately 6.8 million primary library transformants were plated onto plates lacking histidine, leucine, and tryptophan. A total of 148 colonies grew on the histidine dropout plates, 55 of which

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stained positive when tested for expression of β -galactosidase. After three rounds of selection on synthetic minimal (SD) media plates lacking leucine, tryptophan, and histidine, and confirming by the expression of the second reporter gene *lacZ* using a β -galactosidase assay, 47 colonies seemed to be strong interacting candidates.

5

Example 4

Plasmid DNA was extracted from the 47 independent yeast colonies and shuttled into *E. coli* strain HB101, which carries the *leuB* mutation. Therefore, the prey plasmid (cDNA-pGAD 10) was selected for on minimal nutrient plates since pGAD 10 bears the *LEU2* marker.

The 47 independently rescued prey plasmids purified from *E. coli* were re-tested in the yeast two-hybrid system with harpin as bait. They were also tested against unrelated proteins. 25 turned out to be interacting candidates, 20 of which were strong specific interacting candidates. Sequencing analysis showed that the 20 independent cDNA clones were actually from the same gene with different integrity at the 5' end. The sequence reactions were performed using the PE Prism BigDyeTM dye terminator reaction kit. The sequencing gel was run in Thatagen (Bothell, WA)

One of the eight plasmids, which had the longest cDNA insert of 1kb, was used for further analysis. When co-transformed into yeast strain L40, it was shown to be negative with empty bait and unrelated proteins in the Two-Hybrid system, indicating the specificity of the interaction between harpin and this receptor candidate. See Figure 3.

25 Example 5

The longest cDNA insert, HrBP1, was subcloned into the Bam HI and SalI sites of the bait vector pVJL 11. This construct didn't show auto-activation of the reporter genes, nor interaction with unrelated proteins in the yeast Two-Hybrid system. However, the expression of the reporter genes was activated when L40 was co-transformed with HrBP1-pVJL11 and HrpN-pGAD GH, indicating the specific interaction between HrBP1p (the protein encoded by HrBP1) and harpin. See Figure 4.

Example 6

Total RNA was extracted from two-week-old *Arabidopsis thaliana* using QIAGEN RNeasy plant mini kit (Qiagen, Inc., Valencia, California). Poly A⁺ RNA was further purified from the total RNA with a QIAGEN Oligotex column (Qiagen, Inc., Valencia, California). A Northern blot was carried out using the translated region of HrBP1 as a probe. One single species with an apparent molecular weight of about 1.1 Kb was detected from both total RNA and Poly A⁺ RNA.

Therefore, the longest cDNA of HrBP1 from the yeast two-hybrid screen seems to be the full-length cDNA. The integrity of the 5' of cDNA was further confirmed by a primer extension assay.

As described, a yeast Two-Hybrid system was used to screen for harpin interacting proteins. HrpN of *Erwinia amylovora* was subcloned into the yeast Two-Hybrid bait vector pVJL11, which has a *TRP1* marker. The lexA harpin fusion protein is expressed from this construct in yeast. The *Arabidopsis thaliana* MATCHMAKER cDNA library (Clontech Laboratories, Inc., Palo Alto, CA) was screened for hypersensitive response elicitor interacting proteins. 6.8 million independent colonies were screened, and HrBP1 was identified as a strong specific harpin interacting candidate. HrBP1 was mapped to *Arabidopsis thaliana* genomic DNA, chromosome 3, P1 clone MLM24 (Nakamura, "Structural Analysis of *Arabidopsis thaliana* chromosome 3," Direct submission to the DDBJ/EMBL/GenBank databases (1998), which is hereby incorporated by reference). Four exons and three introns were discovered (See Figure 5). Exon 4 includes a 130 bp non-translated 3' region. The in-frame open reading frame from the first methionine encodes a polypeptide (named HrBP1p) of 284 amino acids. The predicted molecular weight of HrBP1p is 30454.3 and pI is 5.72. There is no apparent hydrophobic trans-membrane domain in this polypeptide. SMART Simple Modular Architecture Research Tool (V3.1) predicted the first 18 amino acids as a signal sequence. The HrBP1-AD fusion prey was negative with empty bait and unrelated proteins in the yeast 2-H system, indicating the specificity of the interaction between harpin and this receptor candidate. When being put in the opposite orientation, i.e.

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HrBP1p fused with the DNA-BD and harpin with the AD, they still specifically interacted with each other.

HrBP1 has no significant sequence similarity with sequences deposited in major sequence database accessible with the Blast search program. Therefore,
 5 HrBP1p is a novel protein.

Example 7

The HrBP1 cDNA was subcloned into the Nde I and Sal I sites of the
 10 vector pET-28a (Novagen, Madison, WI). HrBP1p was expressed from this vector in *E. coli* as a His-tagged protein and purified with Ni-NTA resin (QIAGEN Inc., Valencia, CA) according to the manual provided by the manufacturer. This recombinant protein increased harpin's ability to induce HR in tobacco plants. His-tag removed HrBP1 recombinant protein was used to generate anti-HrBP1 antibody to
 15 facilitate biochemical and functional studies of HrBP1. Preliminary localization study using anti-HrBP1 antibody in a Western blot showed that HrBP1p exists everywhere in *Arabidopsis*, including its leaves, stems, and roots.

Example 8

20 10µg of total RNA from 14 different plant species was separated on 1% agarose gel, and then transferred to Amersham Hybond NX membrane (Amersham Pharmacia Biotech, Piscataway, New Jersey). The RNA probe, which was complementary to bases 651-855 of HrBP1 coding region, was generated using
 25 Ambion Strip-EZ RNA kit (Ambion Inc., Houston, Texas). Membrane hybridization was done with Ambion ULTRAhyb (Ambion Inc., Houston, Texas), procedure according to manufacturer recommendation.

The sequence of the HrBP1 fragment used to generate the Northern probe (SEQ. ID. No. 9) is as follows:

30

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gatcaagata acatttgaga aaacaactgt gaagacatcg ggaaacttgt cgcagattcc 60
tccgtttgat atcccagaggc ttcccagacag ttccagacca tcgtcaaacc ctggaactgg 120
ggatttcgaa gttacctatg ttgatgatac catgcgcata actcgcgggg acagaggtga 180
acttagggta ttcgtcattg cttaa                                     205
  
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This Northern blot picked up a band with similar size as HrBP1 in all the plant species tested, including tobacco, wheat, corn, citrus, cotton, grass, pansy, pepper, potato, tomato, soybean, sun flower, and lima bean, which indicated HrBP1-like genes exist universally. See Figure 6.

5

Example 9

HrBP1 homologue from rice, R6, was clone by yeast two-hybrid screening using harpin as bait. It not only interacted with full length harpin but also
10 interacted with a harpin fragment that contains the second HR domain (see Figure 7). However, it is not a full-length cDNA; there is some sequence information missing from the 5' end. The partial sequence of HrBP1-like cDNA from rice encodes a peptide of 203 amino acids, R6-p, which starts at amino acid 84 of HrBP1p. They are 74.4% identical and 87.2% positive at the protein level, they are 65% identical at the
15 DNA level.

The following shows the sequence alignment of HrBP1 (SEQ. ID. No. 1 starting at amino acid 84) and R6 (SEQ. ID. No. 4) at the protein level:

At protein level: Identities = 151/203 (74.4%), Positives = 177/203 (87.2%), Gaps = 2/203 (0%)

20 R6-p : 1 VAALKVKLLSAVSGLNRLAGSQEDLDRADAAARELEAAAGGGPVDLERDVKLQGRWRL
+A LK+KLLS VSGLNRL S +DL+RA+ AA+ELE A GGPVDL D+DKLQG+WRL
HrBP1p: 84 IALLKLLSVVSGLNRLVASVDDLERAEVAAKELETA--GGPVDLTDDLQKQKWL

25 R6-p : 61 VYSSAFSSRTLGGSRPGPPTGRLLPITLGQVFQRIDVVS KD FDNIVDVELGAPWPLPPVE
+YSSAFSSR+LGGSRPG PTGRL+P+TLGQVFQRIDV SKDFDNI +VELGAPWP PP+E
HrBP1p: 142 LYSSAFSSRSLGGSRPGLPTGRLLPITLGQVFQRIDVFS KD FDNIAEVELGAPWPFPPLE

30 R6-p : 121 LTATLAHKFEIIGTSSIKITFDKTTVKTKGNLSQLPPLEVPRI PDNL RPPSNTGSGEFEV
TATLAHKFE++GT IKITF+KTTVKT GNLSQ+PP ++PR+PD+ RP SN G+G+FEV
HrBP1p: 202 ATATLAHKFELLGTCKIKITFEKTTVKTS GNLSQIPPF D IPRLPDSFRPSSNPGTGDFEV

R6-p : 181 TYLDGDTRITRGDRGELRVFVIS 203
TY+D RITRGDRGELRVFI+

35 HrBP1p: 262 TYVDDTM RITRGDRGELRVFVIA 284

The sequence alignment, on a DNA level, of R6 (SEQ. ID. No. 5) and HrBP1 (SEQ. ID. No. 2) starting at nucleotide 265 (i.e. nucleotide 249 of the open reading frame))

40

At DNA level: Identities = 397/610 (65%) (dots indicate identical bases)

45 R6 1 cgtggctgcgctcaaagtcaagcttctgagcgcggtgtccgggctgaaccgcggcctcgc
HrBP1 249 aa.t..atta.....c....at.a..t..t.ta..t..g..at.a...a.a..a..t.t

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	R6	61	ggggagccaggaggatcttgaccgcgcgacgcggcgccgaggctcgaggcgccggc
	HrBP1	309	..c...tgtt...t...t.a..aa.a..t...t...taaa..a..t...aa.t...---
5	R6	121	gggtggcgccccgctgacctggagagggacgtggacaagctgcagggcggtggaggct
	HrBP1	366	---...g..a..g..t..tt.aaccgat..tc.t..t.....t..a...aaa.....
	R6	181	ggtgtacagcagcgcttctcgtcgcgacgctcggcgccagccgccccggccccac
	HrBP1	423	.t....t..t..t.....t..t..t..tt.a..t..t.....t..t..ta..t..
10	R6	241	cggccgcctcctcccatcacctcggcgaggtgtttcagaggatcgatgttgcagcaa
	HrBP1	483	t..a..tt.ga....tg.t..t..t..c.....ac....t.....gt.t....
	R6	301	ggacttcgacaacatcgctcgatgtcgagctcggcgccatggccgctgcccggtgga
15	HrBP1	543	a..t..t..t.....a.ca..g..g..at.a..a..c..t.....at.t.....at.a..
	R6	361	gctgacggcgaccctggctcacaagtttgagatcatcggcacctcgagcataaagatcac
	HrBP1	603	agcc..t.....at....a.....ac..t.a.....t.gc.ag..c.....a..
20	R6	421	attcgacaagacgacggtgaagacgaaggggaacctgtcccagctgccgctggaggt
	HrBP1	663	...t..g..a..a..t.....atc...a...t...g...a..t..t..t..ta..
	R6	481	ccctcgcatcccgacaacctccggcgccgtccaacaccggcgagcgagttcgaggt
	HrBP1	723	...ga.gc.t..c....gtt..a.a..at....a...c..t..a.ct..g..t....a..
25	R6	541	gacctacctcgacggcgacacccgcatacccgcggggacagaggggagctcaggtgtt
	HrBP1	783	t.....tg.t..t.atac..tg.....a..t.....t..a..t....a..
	R6	601	cgtcatctcg
30	HrBP1	843tg.t

Example 10

Arabidopsis thaliana Columbia plants were grown in autoclaved
 35 potting mix in a controlled environment room at a day and night temperature of
 23-20°C and a photoperiod of 14 h light.

A transgenic approach was used for functional analysis of HrBP1.
 Anti-sense HrBP1, which is complementary to SEQ. ID. No. 2, was sub-cloned into
 binary vector pPZP212, and is under the control of NOS promoter. *Arabidopsis*
 40 *thaliana* plants were transformed with this construct via an *Agrobacterium* mediated
 method. The *Agrobacterium tumefaciens* strain used was GV3101 (C58C1 Rif^r)
 pMP90 (Gmr). These antisense lines were designated "as" lines.

Arabidopsis plants were also transformed with a construct, which has
 an inverted repeat with a sense strand of HrBP1 coding region bases 4-650 (i.e. bases
 45 20-666 of SEC. ID. No. 2) and the complementary sequence of bases 20-516 of
 HrBP1 cDNA (i.e. SEQ. ID. No. 2). This construct generated a double-stranded
 mRNA in transformed plants. These transgenic lines were designated "d" lines.

Figure 8 shows the constructs used to transform *Arabidopsis*.

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Both antisense and double-stranded approaches were to silence the expression of HrBP1. The double stranded RNA method was found to be more efficient in silencing the HrBP1 gene. Some transgenic *Arabidopsis* lines showed spontaneous HR-mimic lesion. The most severe line was developmentally retarded, looked very sick, and did not produce seeds.

Example 11

Plants were grown in autoclaved potting mix in a controlled environment room with a day and night temperature of 23-20°C and a photoperiod of 14 h light. 25-day-old plants were inoculated with *Pseudomonas syringae* p.v. tomato DC3000 by dipping the above soil parts of the plants in 10^8 cells ml^{-1} bacteria suspension for 10 second. Seven days after DC3000 inoculation, leaf disks were harvested with cork borer. Bacteria were extracted from leaf disk in 10mM MgCl_2 and plated on King's B agar containing 100 $\mu\text{g/ml}$ rifampicin. Plates were incubated at 28°C for 2 days (Figure 9B) and colonies counted. In Figure 9A, wild type *Arabidopsis* plants had significantly more disease development than transgenic plants. Bacteria counting (Figure 9C) showed that transgenic plants had at least one magnitude less of DC3000 growing inside the leaves. HrBP1 seemed like a negative regulator of plant defense signal transduction pathway in *Arabidopsis*. Its silencing imparted plants with the ability to resist *Pseudomonas syringae* p.v. tomato DC3000.

Example 12

HrBP1 coding region, bases 17-871 of SEQ. ID. No. 2, was sub-cloned into binary vector pPZP212 which is under the control of the NOS promoter (see Figure 10). Tobacco plants were transformed with this construct via an *Agrobacteria* mediated method. The *Agrobacterium tumefaciens* strain used was LBA4404.

Example 13

HrBP1 was over-expressed in tobacco plants under the control of an NOS promoter. Figure 10 shows the construct used for tobacco transformation. Three high expression lines were chosen for further studies in the T2 generation.

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When infiltrated with purified harpin, the transgenic lines developed HR much faster than wild type plants, which is consistent with previous experiment in which His-tagged HrBP1 increased tobacco's sensitivity to harpin protein. The HrBP1 over-expressing lines were about 20-30% taller than wild type Xanthi NN plants (see Figure 11).

Example 14

61-day-old wild type and HrBP1 over-expressing Xanthi NN tobacco plants were inoculated with tobacco mosaic virus by rubbing TMV with diatomaceous earth on the upper surface of leaves. Lesions appeared 2 days after manual inoculation. The picture in Figure 12A was taken 3 days after inoculation. The diameter of disease spots was measured. On average, the diameter of lesions on transgenic plant leaves were 33.4% less than that on wild type plants (Figure 12B). Therefore, the surface area of lesions on transgenic plant leaves was about 44.3% of those of the wild type plants. HrBP1 seemed to be a positive regulator of the plant signal transduction pathway for growth and disease resistance in tobacco.

Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.

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WHAT IS CLAIMED:

1. An isolated protein which serves as a receptor in plants for plant pathogen hypersensitive response elicitors.
- 5 2. A protein according to claim 1, wherein the plant pathogen is selected from the group consisting of *Erwinia*, *Pseudomonas*, *Xanthamonas*, *Phytophthora*, and *Clavibacter*.
- 10 3. A protein according to claim 2, wherein the plant pathogen is an *Erwinia* pathogen.
4. A protein according to claim 3, wherein the plant pathogen is *Erwinia amylovora*.
- 15 5. A protein according to claim 1, wherein the protein is from a monocot.
6. A protein according to claim 5, wherein the protein is from
- 20 rice.
7. A protein according to claim 1, wherein the protein has a partial amino acid sequence of SEQ. ID. No. 4.
- 25 8. A protein according to claim 1, wherein the protein is from a dicot.
9. A protein according to claim 8, wherein the protein is from *Arabidopsis thaliana*.
- 30 10. A protein according to claim 1, wherein the protein has an amino acid sequence of SEQ. ID. No. 1.

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11. A protein according to claim 1, wherein the protein is recombinant.
- 5 12. An isolated nucleic acid molecule encoding a protein according to claim 1.
13. A nucleic acid molecule according to claim 12, wherein the plant pathogen is selected from the group consisting of *Erwinia*, *Pseudomonas*,
10 *Xanthamonas*, *Phytophthora*, and *Clavibacter*.
14. A nucleic acid molecule according to claim 13, wherein the plant pathogen is an *Erwinia* pathogen.
- 15 15. A nucleic acid molecule according to claim 14, wherein the plant pathogen is *Erwinia amylovora*.
16. A nucleic acid molecule according to claim 12, wherein the protein is from a monocot.
- 20 17. A nucleic acid molecule according to claim 16, wherein the protein is from rice.
18. A nucleic acid molecule according to claim 12, wherein the
25 protein has a partial amino acid sequence of SEQ. ID. No. 4.
19. A nucleic acid molecule according to claim 12, wherein the nucleic acid hybridizes to the nucleotide sequence of SEQ. ID. No. 5 under stringent conditions of hybridization buffer comprising 20% formamide in 0.9 M saline/0.09M
30 SSC buffer at a temperature of 42°C.

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20. A nucleic acid molecule according to claim 12, wherein the nucleic acid has a nucleotide sequence comprising SEQ. ID. No. 5.

21. A nucleic acid molecule according to claim 12, wherein the protein is from a dicot.

22. A nucleic acid molecule according to claim 21, wherein the protein is from *Arabidopsis thaliana*.

23. A nucleic acid molecule according to claim 12, wherein the protein has an amino acid sequence of SEQ. ID. No. 1.

24. A nucleic acid molecule according to claim 12, wherein the nucleic acid hybridizes to the nucleotide sequence of SEQ. ID. Nos. 2 or 9 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C.

25. A nucleic acid molecule according to claim 12, wherein the nucleic acid has a nucleotide sequence of SEQ. ID. No. 2.

26. A nucleic acid according to claim 12, wherein the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C.

27. A nucleic acid according to claim 12, wherein the nucleic acid has a nucleotide sequence comprising SEQ. ID. No. 3.

28. An antisense nucleic acid molecule to the nucleic acid according to claim 12.

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29. An expression vector containing a nucleic acid molecule according to claim 12 which is heterologous to the expression vector.

5 30. An expression vector according to claim 29, wherein the nucleic acid molecule is positioned in the expression vector in sense orientation and correct reading frame.

31. An expression vector according to claim 29, wherein either:
10 (1) the protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. Nos. 2 or 9 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (3) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising
15 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (5) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 3; (6) the protein has an amino acid sequence of SEQ. ID. No. 4; (7) the nucleic acid hybridizes to the nucleotide sequence of SEQ. ID. No. 5 under stringent conditions of hybridization buffer comprising 20% formamide in 0.9 M saline/0.09M SSC buffer at a temperature
20 of 42°C; or (8) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 5.

32. An expression vector containing a nucleic acid molecule according to claim 28 which is heterologous to the expression vector.

25 33. A transgenic host cell transformed with the nucleic acid molecule according to claim 12.

34. A host cell transformed according to claim 33, wherein the host cell is selected from the group consisting of a plant cell and a bacterial cell.
30

35. A host cell according to claim 33, wherein the DNA molecule is transformed with an expression system.

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36. A host cell according to claim 33, wherein either: (1) the protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. Nos. 2 or 9 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (3) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (5) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 3; (6) the protein has an amino acid sequence of SEQ. ID. No. 4; (7) the nucleic acid hybridizes to the nucleotide sequence of SEQ. ID. No. 5 under stringent conditions of hybridization buffer comprising 20% formamide in 0.9 M saline/0.09M SSC buffer at a temperature of 42°C; or (8) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 5.

15

37. A host cell transformed with a nucleic acid molecule according to claim 28.

38. A transgenic plant transformed with the DNA molecule of claim 12.

20

39. A transgenic plant according to claim 38, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

25

40. A transgenic plant according to claim 38, wherein the plant is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, *petunia*, *pelargonium*, *poinsettia*, *chrysanthemum*, *carnation*, and *zinnia*.

30

41. A transgenic plant according to claim 38, wherein the plant is a monocot.
- 5 42. A transgenic plant according to claim 38, wherein the plant is from a dicot.
- 10 43. A transgenic plant according to claim 38, wherein either: (1) the protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. Nos. 2 or 9 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (3) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (5) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 3; (6) the protein has an amino acid sequence of SEQ. ID. No. 4; (7) the nucleic acid hybridizes to the nucleotide sequence of SEQ. ID. No. 5 under stringent conditions of hybridization buffer comprising 20% formamide in 0.9 M saline/0.09M SSC buffer at a temperature of 42°C; or (8) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 5.
- 15 20
44. A transgenic plant transformed with a nucleic acid molecule according to claim 28.
- 25 45. A transgenic plant seed transformed with the DNA molecule of claim 12.
- 30 46. A transgenic plant seed according to claim 45, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber,

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apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

47. A transgenic plant seed according to claim 45, wherein the
5 plant is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*,
petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

48. A transgenic plant seed according to claim 45, wherein the
10 plant is a monocot.

49. A transgenic plant seed according to claim 45, wherein the
plant is a dicot.

50. A transgenic plant seed according to claim 45, wherein either:
15 (1) the protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid
hybridizes to a nucleotide sequence of SEQ. ID. Nos. 2 or 9 under stringent
conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M
SSC buffer at a temperature of 42°C; (3) the nucleic acid comprises a nucleotide
sequence of SEQ. ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence
20 of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising
20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (5) the
nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 3; (6) the protein has
an amino acid sequence of SEQ. ID. No. 4; (7) the nucleic acid hybridizes to the
nucleotide sequence of SEQ. ID. No. 5 under stringent conditions of hybridization
25 buffer comprising 20% formamide in 0.9 M saline/0.09M SSC buffer at a temperature
of 42°C; or (8) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 5.

51. A transgenic plant seed transformed with a nucleic acid
molecule according to claim 28.

30

52. A method of identifying agents targeting plant cells
comprising:

- 50 -

forming a reaction mixture comprising a protein according to claim 1 and a candidate agent;

evaluating the reaction mixture for binding between the protein and the candidate agent; and

5 identifying candidate compounds which bind to the protein in the reaction mixture as plant cell targeting agents.

10 53. A method according to claim 52, wherein the protein is from a monocot.

54. A method according to claim 53, wherein the protein is from rice.

15 55. A method according to claim 52, wherein the protein has an amino acid sequence comprises SEQ. ID. No. 4.

56. A method according to claim 52, wherein the protein is from a dicot.

20 57. A method according to claim 56, wherein the protein is from *Arabidopsis thaliana*.

25 58. A method according to claim 52, wherein the protein has an amino acid sequence of SEQ. ID. No. 1.

59. A method of identifying agents targeting plant cells comprising:

forming a reaction mixture comprising a host cell transformed with a nucleic acid molecule according to claim 12 and a candidate agent;

30 evaluating the reaction mixture for binding between protein produced by the host cell and the candidate agent; and

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identifying candidate compounds which bind to the protein produced by the host cell in the reaction mixture as plant cell targeting agents.

- 5 60. A method according to claim 59, wherein the protein is from a monocot.
61. A method according to claim 60, wherein the protein is from rice.
- 10 62. A method according to claim 59, wherein the protein is from a dicot.
63. A method according to claim 62, wherein the protein is from *Arabidopsis thaliana*.
- 15 64. A method according to claim 59, wherein either: (1) the protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. Nos. 2 or 9 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (3) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (5) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 3; (6) the protein has an amino acid sequence of SEQ. ID. No. 4; (7) the nucleic acid hybridizes to the nucleotide sequence of SEQ. ID. No. 5 under stringent conditions of hybridization buffer comprising 20% formamide in 0.9 M saline/0.09M SSC buffer at a temperature of 42°C; or (8) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 5.
- 20 65. A method of enhancing plant receptivity to treatment with hypersensitive response elicitors comprising:
- providing a transgenic plant or transgenic plant seed transformed with the nucleic acid molecule according to claim 12.
- 30

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66. A method according to claim 65, wherein either: (1) the protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. Nos. 2 or 9 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (3) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (5) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 3; (6) the protein has an amino acid sequence of SEQ. ID. No. 4; (7) the nucleic acid hybridizes to the nucleotide sequence of SEQ. ID. No. 5 under stringent conditions of hybridization buffer comprising 20% formamide in 0.9 M saline/0.09M SSC buffer at a temperature of 42°C; or (8) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 5.

15

67. A method according to claim 65, wherein a transgenic plant is provided.

68. A method according to claim 65, wherein a transgenic plant seed is provided and said method further comprises:
planting the plant seeds under conditions effective for plants to grow from the planted plant seeds.

69. A method according to claim 65, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

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70. A method according to claim 65, wherein the plant is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

5 71. A method according to claim 65, wherein the hypersensitive response elicitor treatment is for imparting disease resistance.

72. A method according to claim 65, wherein the hypersensitive response elicitor treatment is for enhancing plant growth.

10

73. A method according to claim 65, wherein the hypersensitive response elicitor treatment is for controlling insects.

15 74. A method according to claim 65, wherein the hypersensitive response elicitor treatment is for imparting stress tolerance.

75. A method according to claim 65, wherein the transgenic plant or plant seed is further transformed with a second nucleic acid encoding a hypersensitive response elicitor, wherein expression of the second nucleic acid effects
20 the hypersensitive response elicitor treatment.

76. A method according to claim 65, wherein the hypersensitive response elicitor treatment comprises:
applying a hypersensitive response elicitor to the plant or plant
25 seed.

77. A method according to claim 76, wherein the hypersensitive response elicitor is applied in isolated form.

30 78. A method of imparting disease resistance, enhancing growth, controlling insects, and/or imparting stress resistance to plants comprising:

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providing a transgenic plant or transgenic plant seed transformed with a DNA construct effective to silence expression of a nucleic acid molecule according to claim 12.

- 5 79. A method according to claim 78, wherein the protein is from a monocot.
80. A method according to claim 79, wherein the protein is from rice.
- 10 81. A method according to claim 78, wherein the protein is from a dicot.
82. A method according to claim 81, wherein the protein is from
- 15 *Arabidopsis thaliana*.
83. A method according to claim 78, wherein either: (1) the protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. Nos. 2 or 9 under stringent conditions of a
- 20 hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (3) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising 20% formamide in
- 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (5) the nucleic acid
- 25 comprises a nucleotide sequence of SEQ. ID. No. 3; (6) the protein has an amino acid sequence of SEQ. ID. No. 4; (7) the nucleic acid hybridizes to the nucleotide sequence of SEQ. ID. No. 5 under stringent conditions of hybridization buffer comprising 20% formamide in 0.9 M saline/0.09M SSC buffer at a temperature of
- 42°C; or (8) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 5.
- 30 84. A method according to claim 78, wherein a transgenic plant is provided.

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85. A method according to claim 78, wherein a transgenic plant seed is provided and said method further comprises:

planting the plant seeds under conditions effective for plants to grow from the planted plant seeds.

5

86. A method according to claim 78, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

10

87. A method according to claim 78, wherein the plant is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

15

88. A method according to claim 78, wherein the transgenic plant or plant seed is further transformed with a second nucleic acid encoding a hypersensitive response elicitor, wherein expression of the second nucleic acid effects a hypersensitive response elicitor treatment.

20

89. A method according to claim 78 further comprising: applying a hypersensitive response elicitor to the plant or plant seed.

25

90. A method according to claim 89, wherein the hypersensitive response elicitor is applied in isolated form.

30

91. A method according to claim 78, wherein disease resistance is imparted to plants.

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92. A method according to claim 78, wherein enhanced growth is imparted to plants.

93. A method according to claim 78, wherein insect control is
5 imparted to plants.

94. A method according to claim 78, wherein stress resistance is imparted to plants.

10 95. A method according to claim 78, wherein the DNA construct is an antisense nucleic acid molecule to a nucleic acid molecule encoding a receptor in plants for plant pathogen hypersensitive response elicitors.

15 96. A method according to claim 78, wherein the DNA construct is transcribable to a first nucleic acid encoding a receptor in plants for plant pathogen hypersensitive response elicitors coupled to a second nucleic acid encoding the inverted complement of the first nucleic acid.

20 97. A method of imparting disease resistance, enhancing growth, controlling insects, and/or imparting stress resistance to plants comprising:
providing a transgenic plant or transgenic plant seed transformed with the nucleic acid molecule according to claim 12.

25 98. A method according to claim 97, wherein either: (1) the protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. Nos. 2 or 9 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (3) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3
30 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (5) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 3; (6) the protein has an amino acid

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sequence of SEQ. ID. No. 4; (7) the nucleic acid hybridizes to the nucleotide sequence of SEQ. ID. No. 5 under stringent conditions of hybridization buffer comprising 20% formamide in 0.9 M saline/0.09M SSC buffer at a temperature of 42°C; or (8) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 5.

5

99. A method according to claim 97, wherein a transgenic plant is provided.

100. A method according to claim 97, wherein a transgenic plant
10 seed is provided and said method further comprises:
planting the plant seeds under conditions effective for plants to
grow from the planted plant seeds.

101. A method according to claim 97, wherein the plant is selected
15 from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower,
peanut, corn, potato, sweet potato, bean pea, chicory, lettuce, endive, cabbage, brussel
sprout, beet, parsnip, turnip, cauliflower, broccoli, radish, spinach, onion, garlic,
eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear,
melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato,
20 sorghum, and sugarcane.

102. A method according to claim 97, wherein the plant is selected
from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium,
poinsettia, chrysanthemum, carnation, and zinnia.

25

103. A method according to claim 97, wherein disease resistance is
imparted.

104. A method according to claim 97, wherein plant growth is
30 enhanced.

- 58 -

105. A method according to claim 97, wherein insects are
controlled.
- 5 106. A method according to claim 97, wherein stress tolerance is
imparted.
107. A method according to claim 97, wherein the protein is from a
monocot.
- 10 108. A method according to claim 107, wherein the protein is from
rice.
109. A method according to claim 97, wherein the protein is from a
dicot.
- 15 110. A method according to claim 109, wherein the protein is from
Arabidopsis thaliana.

SEQUENCE LISTING

<110> Eden Bioscience Corporation

<120> RECEPTORS FOR HYPERSENSITIVE RESPONSE ELICITORS AND
USES THEREOF

<130> 21829/63

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          50             55             60

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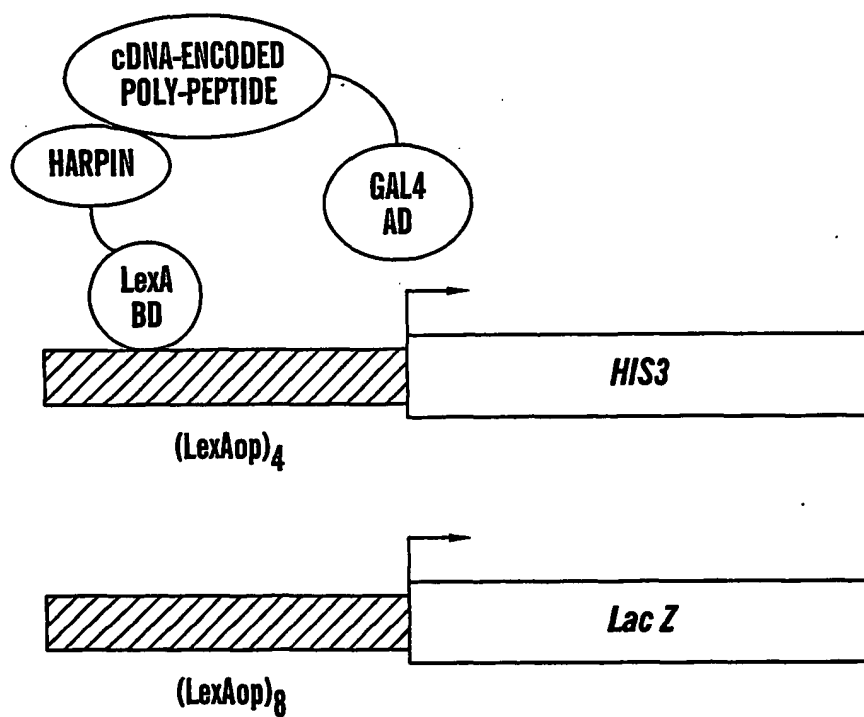
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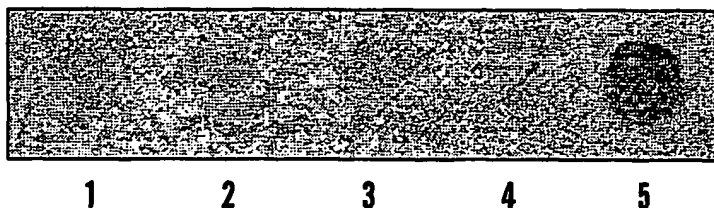
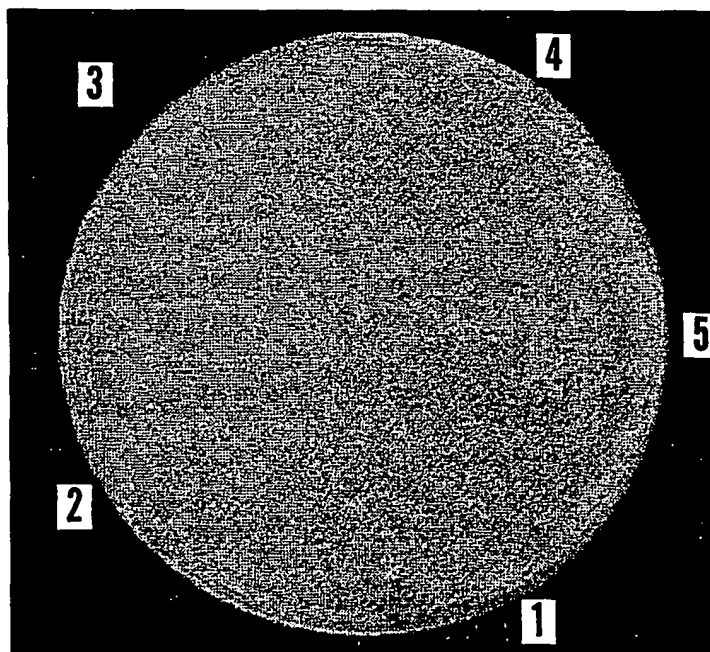
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1/12

**FIG. 1**

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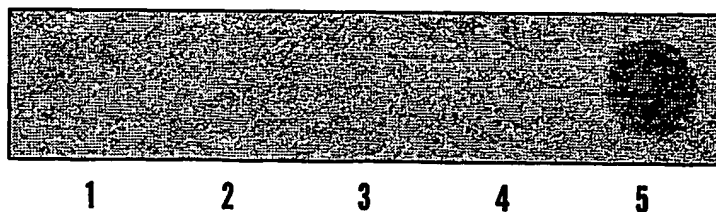
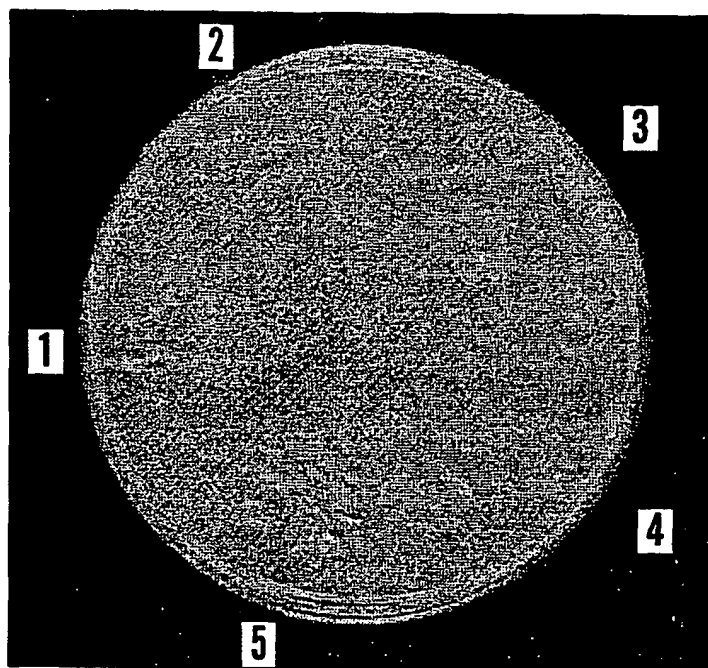
**FIG. 2A**

	BAIT	PREY
1	HARPIN	EMPTY VECTOR
2	HARPIN	UNRELATED PROTEIN
3	EMPTY VECTOR	HARPIN
4	UNRELATED PROTEIN	HARPIN
5	(POSITIVE CONTROL)	

FIG. 2B

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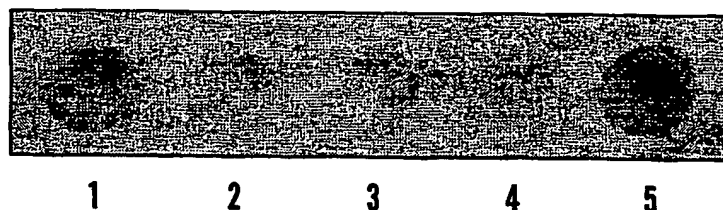
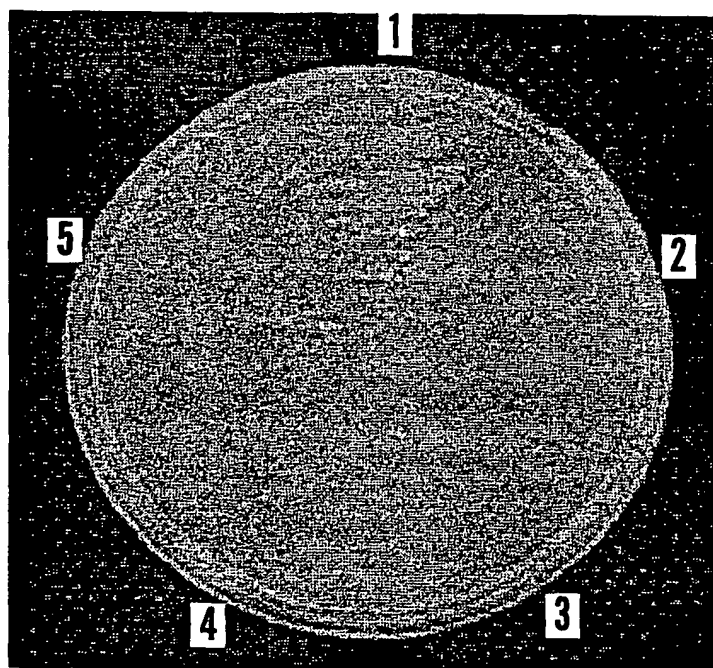
**FIG. 3A**

	BAIT	PREY
1	HARPIN	HrBP1
2	UNRELATED PROTEIN 1	HrBP1
3	UNRELATED PROTEIN 2	HrBP1
4	EMPTY VECTOR	HrBP1
5	(POSITIVE CONTROL)	

FIG. 3B

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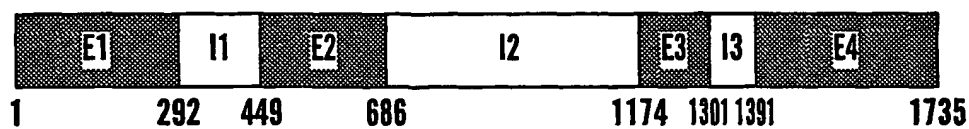
**FIG. 4A**

	BAIT	PREY
1	HrBP1	HARPIN
2	HrBP1	EMPTY VECTOR
3	HrBP1	UNRELATED PROTEIN 1
4	HrBP1	UNRELATED PROTEIN 2
5	(POSITIVE CONTROL)	

FIG. 4B

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GENE STRUCTURE OF HrBP1

E: EXON

I: INTRON

FIG. 5

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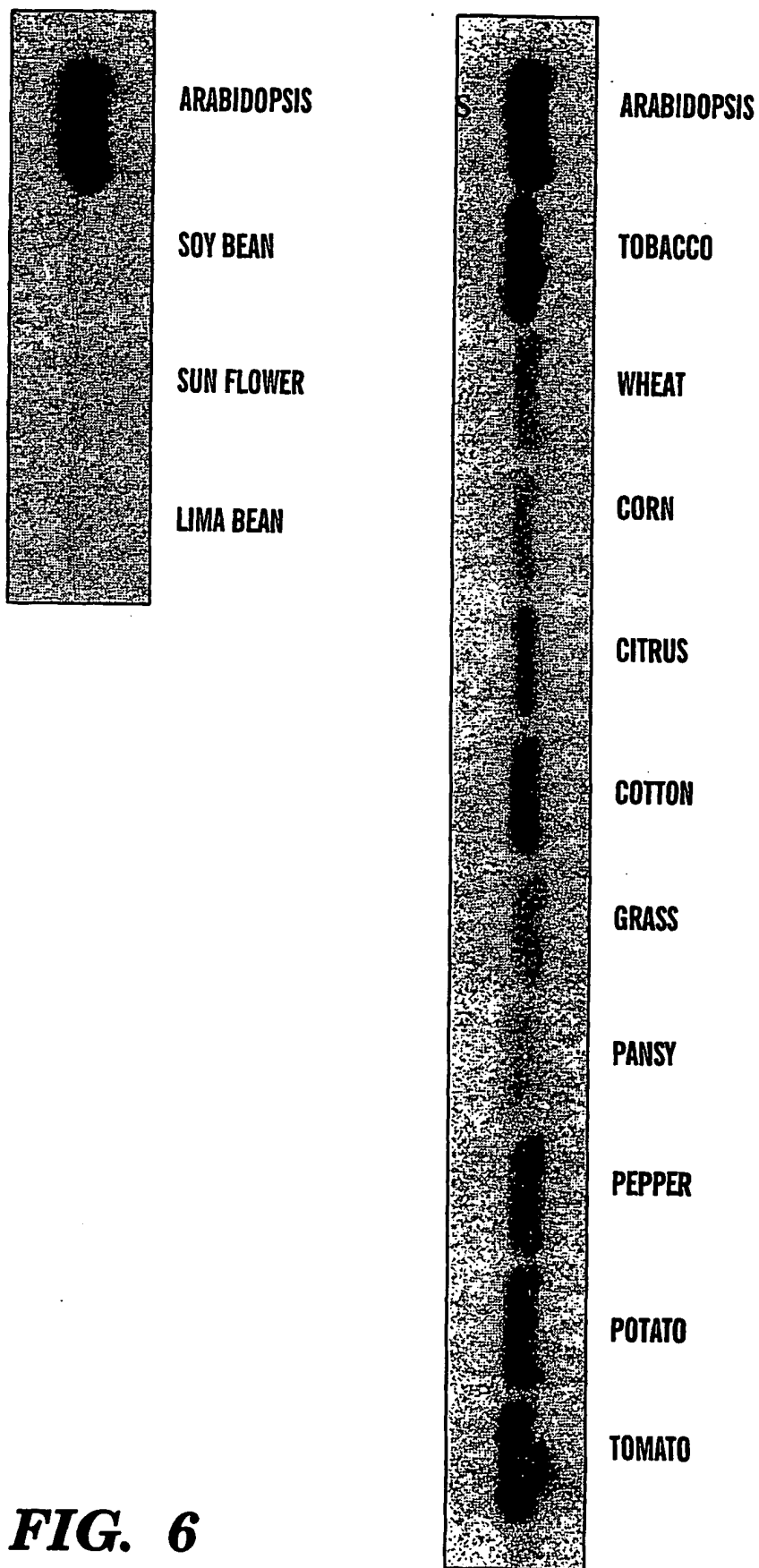
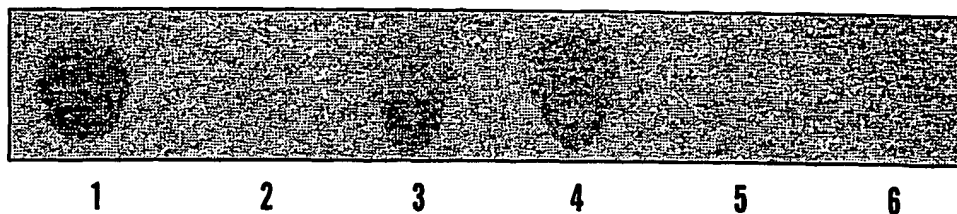
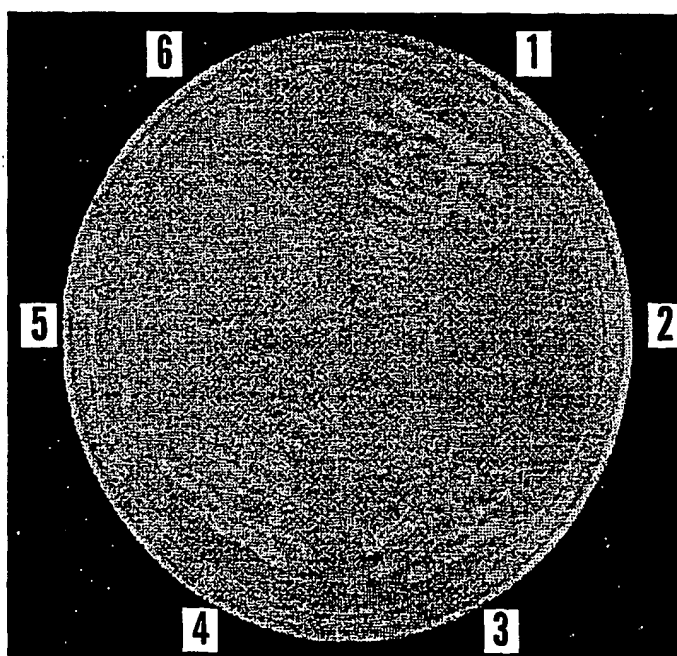


FIG. 6

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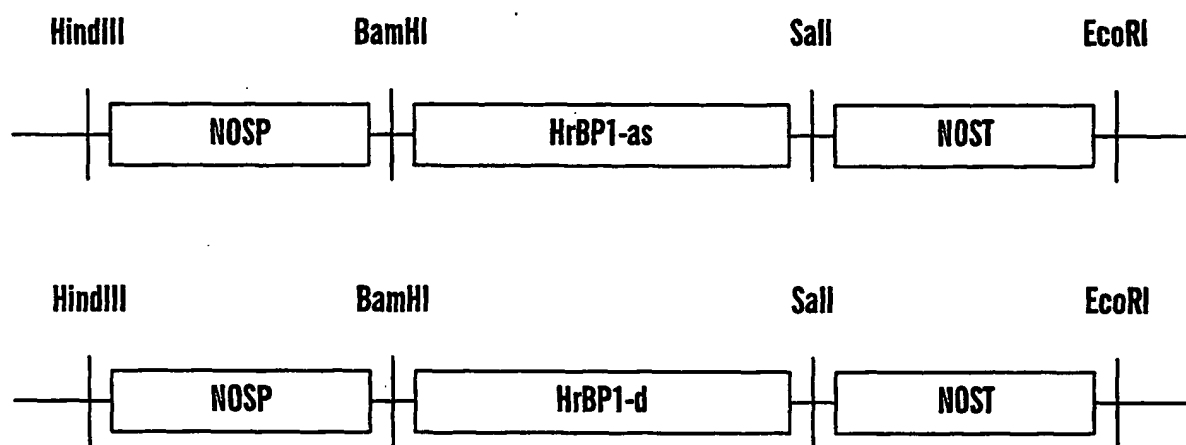
**FIG. 7A**

	BAIT	PREY
1	POSITIVE CONTROL	
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3	hrpN FULL LENGTH	R6
4	hrpN 137-180aa	R6
5	hrpN 210-403aa	R6
6	lexA-lamin	R6

FIG. 7B

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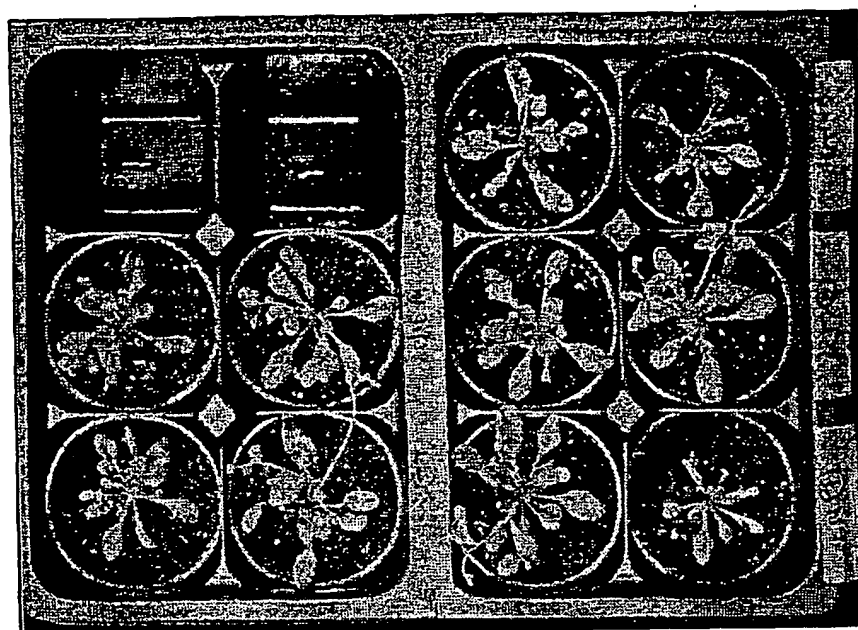


HrBP1-d: HrBP1 CODING REGION BASES 4-650 SENSE STRAND + 4-500 ANTI-SENSE STRAND

FIG. 8

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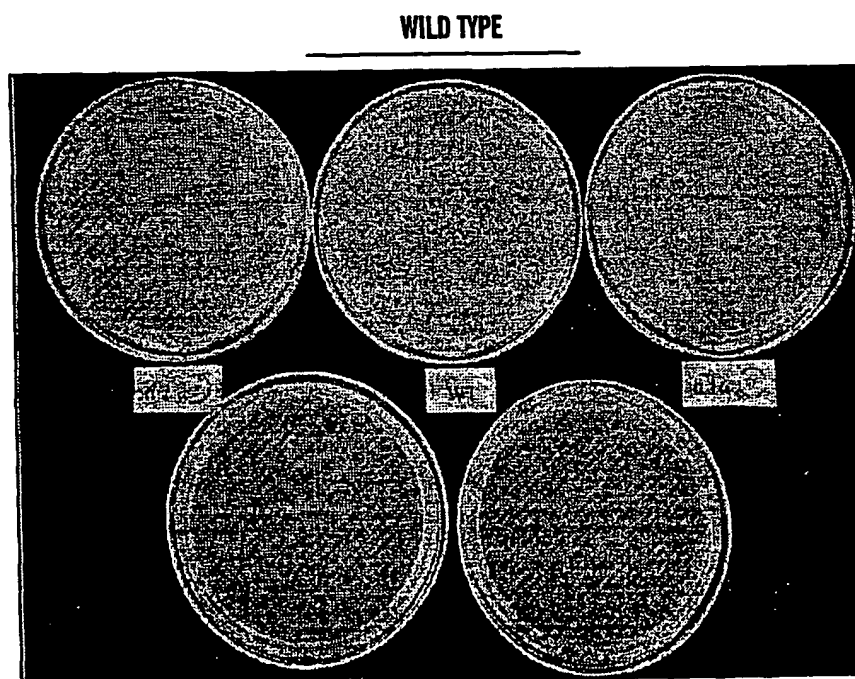
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WILD TYPE

TRANSGENIC LINES

FIG. 9A



WILD TYPE

FIG. 9B

	cfu/cm ²
WILD TYPE	5.5 X 10 ⁷
d2-2	2.0 X 10 ⁵
d16-9	2.0 X 10 ⁵
as14-7	2.1 X 10 ⁶
as17-8	4.1 X 10 ⁶

FIG. 9C

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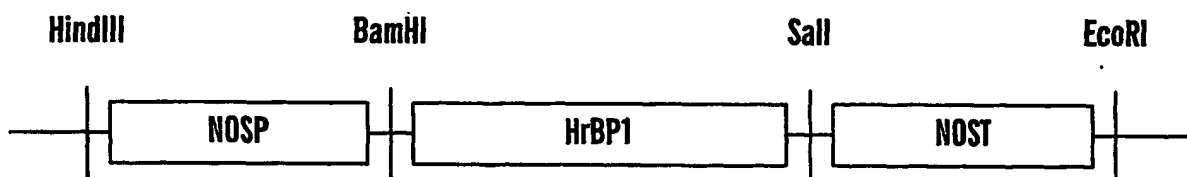
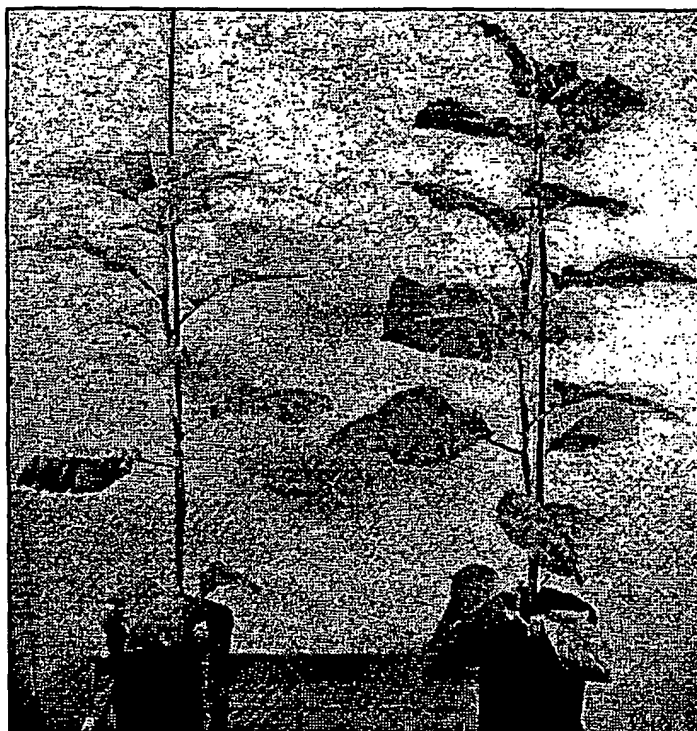


FIG. 10

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WILD TYPE

HrPP1 OVER-EXpressING
TOBACCO**FIG. 11A**

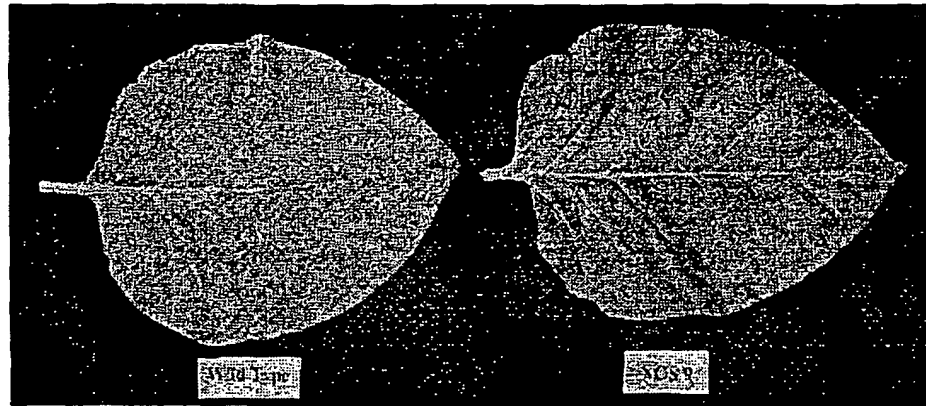
HEIGHT OF T2 TOBACCO PLANTS AFTER 52 DAYS TRANSFERRING TO SOIL

WILD TYPE	49.5 ± 3.2 cm
LINE 1	59.5 ± 3.8 cm
LINE 2	61.7 ± 1.9 cm
LINE 3	64.3 ± 5.5 cm

FIG. 11B

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WILD TYPE

HrBP1 OVER-EXPRESSING LINE

FIG. 12A

	DIAMETER OF DISEASE SPOT
WILD TYPE	$2.97 \pm 0.49\text{mm}$
LINE 1	$1.97 \pm 0.31\text{mm}$
LINE 2	$1.95 \pm 0.37\text{mm}$
LINE 3	$2.01 \pm 0.33\text{mm}$

FIG. 12B

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